IMPERIAL BUREAU OF
HORTICULTURE AND PLANTATION CROPS

TECHNICAL COMMUNICATION No. 10

PLANT INJECTION FOR DIAGNOSTIC AND CURATIVE PURPOSES

By
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October, 1938.

Price 5/-

Published by the Imperial Bureau of Horticulture and Plantation Crops, East Malling, Kent, England

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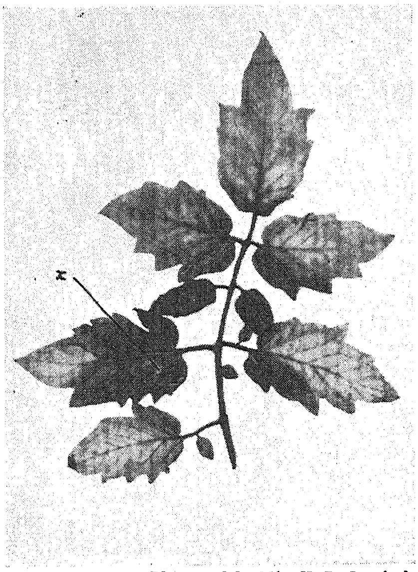
Photograph by Miss K. E. Cornford.



Photograph by Miss K. E. Cornford.

FIG. 1.
The effect of injecting urea into a young broad bean leaf through an incision just below the arrows, which indicate the limits of permeation of the urea.

FIG. 2.
The effect of injecting sodium borate through th incision x into a young tomato leaf. Note ho widespread is the effect compared with that of ure in Fig. 1.

Plate 2

Photograph by Mr. J. Amos.

Effect of the injection of 0·1 per cent. ferric chloride into a shoot of chlorotic peach tree. The two terminal leaves and half of the third have regained their normal colour.

PLANT INJECTION FOR DIAGNOSTIC AND CURATIVE PURPOSES

CONTENTS

FOREWORD

By PROFESSOR V. H. BLACKMAN, Sc.D., F.R.S., Director of the Research Institute of Plant Physiology, Imperial College of Science and Technology, London.

This monograph like the earlier one on incompatibility has an appeal beyond economic biology. It is a truth rather humbling to plant physiologists that in spite of all the advances they have made in recent years the full secret of the mechanism of water movement in the plant is not yet disclosed. Much knowledge has been gained, however, and the whole technique of injection—here for the first time fully developed—does in fact depend on a physiological peculiarity of the water-system of the plant. Whereas the channels of the blood-system of the animal show in the main a positive pressure and discharge fluid when laid open, those of the plant water system are in general under a negative pressure, and show not bleeding but suction when severed. Fluid supplied to an artificial opening in the channels is thus drawn in.

The development of this special technique has in its turn led to observations of general physiological interest, such as those relating to the "leaf pattern" on injection. This is here shown to depend both upon the arrangement of the leaves on the stem and the vascular anatomy of the plant.

In tracing the history of the subject the author gives us an attractive account of the early but fumbling uses of plant injection, the first reference going back to Arab sources in the twelfth century. Nothing can then be traced until the fifteenth century when we find plant injection described in the notebooks of that almost universal genius, Leonardo da Vinci. It is a galling thought that we shall never know if any use was made of the fruits which were rendered poisonous by the technique of arsenic injection which Leonardo had devised.

Dr. Roach in the descriptive part of this communication gives the results of his seven years most fruitful study of plant injection. He has developed it into a precise technique which can be followed by others; he has thus placed in our hands a new instrument of great value in both pure and applied biology.

The advantage, what one may call the elegance of the method, lies in the direct application of substances to single organs, i.e. to a leaf or a small part of a leaf, to a branch or a root. As a result many experimental complications are immediately resolved. If a substance is supplied to the soil then all or part of it may then be held back, or it may release other substances from the soil which enter the plant with it or in place of it. Furthermore, while the substance in question is being carried upwards in the plant it may produce changes *en route* which confuse the interpretation of the results. By the injection method all these difficulties are avoided.

To the horticulturist the method is unrivalled in the speed with which in some cases the conditions of deficiency may be elucidated; a diagnosis may even be available in a few days. For remedial measures also the advantages are plain. The curative substances can be used without the loss, alteration or delay in action so often found with applications to the soil.

The plant physiologist, the experimenter in horticulture, and the fruitgrower are thus all in debt to the East Malling Research Station for this important development of its work.

INTRODUCTION

Definition of injection

The term injection is used in the medical, veterinary and zoological sciences in its correct etymological sense, viz. to indicate the forcing of a fluid into the body. Botanists use the term, however, in wider senses. Thus, when the intercellular spaces in a plant tissue become filled with sap in place of air, as for example in the trouble occurring occasionally in apples known as " glassiness " or " water-core ", the affected tissue is said to be injected. When a liquid is introduced into a plant through a cut or a hole in one of its organs, this also is called injection, even when little or no force is used. Again, a plant may be injected without recourse to wounding of any kind. Thus, if a leafy stem be bent down under the surface of water in a convenient container, water will pass in through the leaves, and the tissues will become injected. Finally, if solid substances are introduced into holes or cuts in a plant organ, such treatment has in recent years been termed " solid injection ". In the present paper the term injection will be used to indicate the introduction, by various methods, of liquids and solutions into plant organs, whether under pressure or not, and their spread therein. Work on solid " injection ", however, is not dealt with in it at any length.

History

Early work

Tree injection has been practised since very early times. Ibn-Al-Awam*, writing in the twelfth century, quoted from a work written by Hadj de Granade in 1158, which described methods for imparting perfumes, flavours and medicinal qualities to fruits, and a yellow or blue colour to roses. The methods consisted either in splitting the root or shoot, removing the pith, and introducing suitable substances into the pith cavity, or in inserting such materials between the carefully lifted bark and the wood. The substances included musk, cloves, saffron, etc., in finely pulverized form. It is clear from this that solid " injection " was tried at least as early as the twelfth century.

No further reference to plant injection can be traced until the fifteenth century, when Leonardo da Vinci made an entry in his note books of which the following is a translation†:

> " Boring a hole in a tree with a gimlet and inserting in it arsenic and realgar refined by sublimation and dissolved in boiling water is capable of rendering the fruit of the tree poisonous or of making it wither. The hole must be large and must go right through to the pith and should be made when the fruits are ripening. The poisonous liquid should be squirted in with a syringe and the hole should be plugged tight with wood. The operation can also be carried out when the sap is rising in the trees."

The wording of this entry suggests that Leonardo da Vinci carried out systematic experiments and that the resulting method was devised for practical use. This is perhaps the earliest reference to liquid injection.

Methods similar to those described by Hadj de Granade were published anonymously in 1602 in *The Orchard and Garden*‡ as follows :

> . . . " well smelling and spiced fruit. Cleave a tree asunder or a branch of a fruitful tree, to the heart or pith, and cut a piece out of it, and put therein powdered spices, or whatsoever you will, or what colour you will desire, and tie a barke hard about it and anoint it with lome and oxe dung, and the fruit will get both the savour and colour, according to the spice you have put in it.
>
> *How sower fruits be made sweet.*
>
> Which tree beareth sower fruits, in the same pierce a hole a foot or somewhat lesse above the root and fill that with honey, and stop the hole with a haw-thorne braunch, and the fruit will be sweet.
>
> . . . . .

* The writer is indebted to Prof. R. A. Fisher for this reference.
† The writer is indebted to Mr. D. Akenhead for the translation from the Italian.
‡ The writer is indebted to Mr. R. M. Greenslade for this reference.

How the wormes are to be killed, if they be already grown into the tree.

If you will kill the wormes, which grow into the tree, take pepper, laurell, and incense, and mingle all well together with good wine, and pierce a hole into the tree downward, till to the pith or heart of the tree, and poure this mixture into it, and stop it with a hawthorne, and the wormes will die."

As stated above, this passage bears a general resemblance to that in Ibn-Al-Awam's book, but the idea of killing "worms", i.e. wood-boring insects, is fresh. This and other minor differences suggest that during the intervening five centuries the possibilities of solid "injection" were at least thought about, if not tested experimentally. The descriptive subtitle to this later work: "Gathered from the Dutch and French", and the fact that it was published in London, suggest that the idea of plant injection may have been widely distributed.

A further development of the idea is contained in J. M. Wilson's *The Rural Cyclopædia*:

"A curious method of destroying insects on trees and shrubs, by means of mercury, was invented in 1765 by a person in Hereford, and was speedily adopted by members of the Society for the Encouragement of Art. The inventor pierced the branch of a plumtree slopingly with an awl through the bark and part of the wood, but not to the pith, and poured into the hole a drop or two of mercury, and stopped it up with a small wooden plug; and he found that, on the next day, all the insects dropped off from that branch, and, in a day or two more, from all the other branches, and that the tree continued in full vigour, and throve well throughout the season."

These early attempts, though interesting historically, appear to have been the results of more or less inspired guesses, and the efficacy of most of the methods used may well be doubted; but the idea behind them, like that behind modern injection work, was to improve the health of the plant or to bestow on it special qualities without either killing or even unduly damaging it.

The knowledge available at the time hardly allowed these early experimenters to attain their aims, and no further attempts seem to have been recorded for about a hundred years. During the nineteenth century developments occurred in more than one branch of science which supplied much of the information necessary for new and more successful injection work; and as soon as this had become available a wave of injection experimentation swept more or less simultaneously through Russia, Germany, America and France. This new and more scientific development of plant injection was mainly the indirect result of the attempts of plant physiologists to elucidate the cause of the ascent of sap in trees; and in spite of the lack in plants of any circulatory system comparable to that for blood in animals, further impetus was probably given by the striking results obtained on animals and man by medical workers towards the end of the century through the use of injection methods. The first act of importance in the scientific development of plant injection was recorded by Magnol (1709), namely, that certain coloured solutions are absorbed by the cut stems of twigs immersed in them, and pass up the stems into the leaves and flowers, the path followed being clearly revealed by the colour. Magnol actually used the method for tracing the path followed by the transpiration stream, a matter which will not be considered in the present paper.

Nearly a century later, Meyer (1808), by cutting off the top of a small tree and submersing the stump in a dye solution proved that the dye penetrated all the roots, reaching all except the thinnest rootlets.

In 1840, Boucherie patented and published methods for causing trees whilst still alive to absorb solutions which would preserve their timber when felled. The liquids killed the trees and the methods used were too drastic to be applied to trees which were not intended for practically immediate felling. The interest of the methods in the present connexion is that they proved that living trees will absorb large volumes of certain solutions when they are brought into contact with cut woody tissues.

The next big advance was made by Hartig (1853) who bored four holes to meet crosswise in the centre of a tree a little above ground level. He closed three of the openings and into the fourth inserted a wooden tap, to which was connected an upright funnel. Into this he poured a solution of pyrolignite of iron, and this became absorbed rapidly. Thus, Hartig seems to have been the first to lead liquid from a reservoir into a hole made in a tree, in this way adapting da Vinci's method for injecting large volumes of liquid. Later, the tree was felled and cut into cross sections. In each section the tissues directly above the holes were blackened in the

form of a cross but the rest of the wood was not discoloured. The dark cross was plainly visible even at a height of 40 ft. above the holes. Hartig deduced from these facts that the movement of the liquid had been confined to the blackened tissues. This probably erroneous conclusion will be considered again later.

McNab (1871, 1875) proved spectroscopically by experiments similar to those of Magnol that metals ascended amputated living branches at different rates when their cut ends were placed in solutions of metallic salts, viz.: lithium 12·8, thallium 7·5 and caesium 3·3 inches in one hour. A few years later Pfitzer (1877) by similar experiments, showed that water travels more quickly than the lithium salt dissolved in it, and he made the further important observation that the rate of travel of the injected lithium solution was greater than could be accounted for by mere diffusion.

While these and other plant physiologists, by experiments designed to throw light on the problem of the ascent of sap, were unconsciously evolving methods which were later to be used for developing tree injection methods, yet another influence began to be felt. Erhart (1873) in 1872 tried to inject plants with a hypodermic syringe as used for human and animal injections. He inserted the needle into the surface layers of soft plant tissues for a distance up to 15 mm., and pressed the piston as he withdrew the needle. In this way the hole made by the needle became filled with the liquid in the syringe. This liquid, of the order of 0·05 c.c. in volume, contained 0·00125 g. of dissolved salt. As in McNab's slightly earlier experiments, subsequent spectroscopic tests proved that some of the salts injected (e.g. lithium chloride, copper sulphate) had travelled into undamaged tissues of the plant both upwards and downwards. The fact that the injection of certain liquids was followed by damage to tissues at some distance from the point of injection was also in harmony with this conclusion. Thus, Erhart made use of the damage produced by a substance to trace its path within the injected plant, a method which has been used in a modified form in the investigations to be described in the present paper.

Sachs (1878), in experiments similar to those of Magnol, McNab and Pfitzer, used both dyes and simple inorganic salts. He noted that whereas a lithium salt travelled up the stem nearly as rapidly as the water in which it was dissolved, substances which dyed the cell walls rose much more slowly. This slowing down of the movement of many dyes as they pass through living or dead plant tissues or capillary spaces, such as those in blotting paper, was studied in great detail by Goppelsroeder (1889, 1901), and some of the methods to be mentioned later for selecting the most suitable dyes for injection experiments were first described by him.

Work published since 1880

It will be noticed that, with the possible exception of those of Erhart, most of the injection experiments so far alluded to were carried out in the hope that the substance injected would merely add its own properties to those of the plant or part of the plant injected. For instance, cloves to give their flavour to the apples borne on the injected tree; plant dyes to give their colour to the flowers borne on the injected tree; preservatives to give their properties to the injected timber. But after plant physiologists had used injection methods for some time in the attempt to study the ascent of sap they began to realize the possibilities of introducing nutrient substances by this method. Thus, Sachs, who had published a paper on the ascent of sap in 1878, published in 1886 an account of experiments in which ferrous sulphate and ferrous chloride solutions were injected into chlorotic acacia trees. His method was a simplified form of the one employed by Hartig. A hole, 1·5 cm. in diameter, was bored below the affected branch, through the bark and sapwood, until the heartwood was reached. The tube of a funnel bent at right angles was fixed in the hole through a bored cork, and the solution was poured down the funnel into the hole in the trunk in such a way that the air in the hole was displaced as the solution took its place. Sachs noticed that only those leaves on branches vertically above the funnel became green. Ten years later Tschermak (1896) described a similar method, apparently in ignorance of Sachs' earlier work.

From this time onward there was a comparatively rapid development of ideas, both in regard to the actual methods used and as to the purposes for which they could be employed;

and in this development national trends of thought can be recognized, which will now be briefly reviewed.

Russian work

An idea developed by Shevyrev (1894, 1903)*, had a great influence on the development of injection methods, but how far it was based on fact cannot even now be accurately assessed. This was that the access of air to the exposed tissues must be rigidly prevented, and Shevyrev suggested that neglect of this precaution was the cause of the failure of others to induce penetration of liquid into tissues other than those directly above the cut wood vessels. (But see p. 55). The two methods he devised to fulfil this purpose have been used by a number of later workers.

The first consisted in driving into the bark the sharpened end of a metal tube, provided with a side tube for leading in the liquid from a reservoir connected to it, the other end of which was closed with a cork through which passed the shank of the boring tool. The tube was kept filled with liquid while the hole was being drilled so that no air could reach it.

His second method consisted in bending pieces of sheet metal into the shape of a half funnel, the edges of which were tacked to the smoothed trunk of the tree and made staunch with putty. The reservoir thus formed was filled with the liquid and incisions were made beneath its surface with a chisel. Roth (1896), in work carried out in Germany apparently in ignorance of Shevyrev's paper, far from excluding air, left his injection hole exposed to the air for 36 hours before admitting the liquid, and he found that absorption was remarkably rapid. His method consisted in drilling a hole, varying according to the size of the tree from 1 to 2·5 cm. in diameter, in slightly sloping fashion downwards into the trunk and passing about two-thirds of the way through it. A tube was cemented into this hole which, after the cement had set, was filled with liquid and connected by rubber tubing with a reservoir attached to the tree a little above the hole.

Shevyrev's chief experiments were carried out mainly with dyes to establish general principles; but he also injected a grape vine with copper sulphate presumably to see what the effect on attack by mildew would be, but he did not give the result. He noticed that the most rapid absorption took place at the beginning of the injection, that the rate of uptake decreased gradually and that it ceased after from three to five days, this being due, in his opinion, to choking of the conducting tissues. He noted that the rate of uptake was influenced by the weather and was lower by night than by day. He does not appear to have attained the main object of his experiments, namely the destruction of insects burrowing just beneath the bark. He believed, however, that fungus diseases could be cured by this method.

According the Jaczewski (1910), Nicolaev-Tzygankov (1898), treated chlorotic trees successfully by introducing powdered ferrous sulphate into holes drilled to the centre of the stem. Reshko (1903) used the same method in 1901 on one thousand chlorotic trees, but the distribution of the salt was irregular, with the result that a number of branches escaped treatment.

Mokrzecki, a Pole working in the Crimea (1903a, b, 1904a, b, c)† also carried out extensive injection experiments. In his paper published in Germany he called the treatment: "Die innere Therapie der Pflanzen". He employed both a slight modification of Shevyrev's method for liquids, and the one used by Nicolaev-Tzygankov (commonly named after Mokrzecki) for solids. He injected more than 500 trees by Shevyrev's method, employing solutions of nutrients varying in concentrations from 0·01 to 0·1% until no more was absorbed. He treated chlorotic trees with either 12 g. of dry ferrous sulphate (for a tree 16-25 cm. in diameter) or with 0·05-0·25% ferrous sulphate solution. This solution caused leaf scorch, which appeared on the third day, starting at the veins. The green colour began to appear four days after injection and no trace of chlorosis was left after ten days. Three weeks later the foliage was dark, glossy and healthy. He also used powdered dry iron pyrophosphate for the same purpose. This treatment and the injection of Knop's and other inorganic nutrient solutions appeared to control infestation by

* The writer is indebted to Mr. H. P. Gould, U.S. Dept. Agriculture, Washington, for locating, and to Mr. C. Audley Richards, of Madison, Wisconsin, for supplying a translation of these two papers.

† The writer is indebted to Dr. J. Majewsky for some of these references and for checking the account of Mokrzecki's work.

the scale insects *Diaspis fallax* on pears and *Mytilaspis pomorum* on apples. Gummosis of apple, pear and other trees was cured by injections of 1% salicylic acid solution. He also attempted to control bark beetles and other insects by similar means. He illustrates a tree that was treated with iron pyrophosphate on one side only, as a result of which the treated side became healthy, in marked contrast to the other side which remained chlorotic and unhealthy.

Pachossky (1903) also published a paper on the subject at about the same time. Jaczewski (1910) in 1909 obtained improvement in growth and general appearance of some of the fruit trees treated, especially stone fruit varieties, by the injection of dry salts corresponding to Sorauer's nutrient solutions. In most cases he used 1 hole, 2 cm. deep by 1.5 cm. across, inserting 4 g. salts, but for larger trees 2 holes with 4 g. in each.

In 1903 Dementiev (1914), in ignorance of the work of his fellow countrymen and of Goff (to be mentioned later), started injecting various poisonous solutions through cuts made in roots, leaf stalks and leaves of plants in attempts to make them distasteful to or unsuitable as food for insects. His final plan resembled Goff's root-stump method. He found that the rate of uptake could be accelerated by increasing the injection pressure and that pressures up to 6 atmospheres could safely be employed on fruit trees.

French work

In France, also, injection methods were being developed about this period. Thus, Mangin (1898) criticized unfavourably the attempts of an engineer called Berget to nourish vines and protect them from fungus diseases by injecting salts. These trials appear to be the earliest instances of true injection mentioned in the literature of that country ; but no original publication dealing with them has been traced. This work was soon followed by the purely physiological experiments of Ray (1901) who injected liquids, including solutions derived from micro-organisms, into leaves through capillary tubes, his aim being to make the leaves immune from disease. Simon (1906) reported injection experiments which had been in progress since 1893 on cider apples, peaches, pears, potatoes, melons, cabbages, etc. The liquid was held in a reservoir at a height of about 2 m. above ground level, and was led through rubber tubing into a tapered wooden or glass tube inserted into a hole bored into the stem of the tree just above that level, the joint being made water-tight with mastic. He reported markedly good results following the injection of a liquid having the same composition as plant sap. He obtained 50 to 100% increases of crop following the injection of sea salt solution into potato plants and an improvement in flavour and quality both in these potatoes and in similarly treated cabbages and cauliflowers.

Fron (1909), using Simon's method, injected solutions of iron sulphate and calcium nitrate into chlorotic pear trees, growing in calcareous soil. The resulting increase in vigour did not extend to the whole of the injected tree and Fron did not consider the method commercially practicable. Opoix (1910), on the other hand, obtained good results from work carried out between 1905 and 1910, in which powdered iron sulphate was introduced into a hole of diameter equal to one-tenth of that of the stem and reaching the pith. This hole was filled with the powdered salt up to, but so as not to touch, the bark, and it was sealed with mastic. Thus, for the first time the size of hole and the dose were made dependent on the size of the tree, the volume of the hole and consequently the amount of iron sulphate being proportional to the cube of its diameter. He found that a second dose in another hole was often necessary to complete the cure. He recommended that the operation be carried out during the period May to July. He reported that trees treated five years previously were still in good condition. Coffigniez (1910), at about the same time, reported equally successful results obtained by him over many years by a method similar to that described by Opoix. Rivière and Bailhache (1910) also published a paper on the same subject.

Passey (1910) at the same time made the interesting observation that chlorotic trees were actually richer in iron than healthy ones and richer even than those which had been cured by injection with iron sulphate. Apparently the greater part of the iron content of the chlorotic tree was in a form of no use to the plant ; this useless iron would be " diluted " by the increased growth resulting from the injection of a small quantity of iron in a form of use to the plant. Arnaud (1919) mixed the ferrous sulphate with olive oil, but with no apparent advantage.

This succession of papers on the cure of chlorosis was momentarily interrupted by one Raybaud (1921) who made unsuccessful attempts to control insect attacks by inserting potassium ferrocyanide powder in holes in fig trees, pines and privet. But the next paper, by Chabrolin (1924) once more concerned chlorosis. His injection of iron sulphate into peach trees resulted in a partial restoration of foliage colour but caused too much damage around the hole to warrant its adoption commercially.

Joessel and Lidoyne (1936) and Joessel, Lidoyne and Pampillon (1937) reported a series of attempts to use injection methods for the cure of chlorosis of fruit trees on a commercial scale. In general they preferred such methods to the application of iron salts to the soil, because large amounts must sometimes be applied to the soil and consequently there is a risk of damaging the roots by an excessive dressing. They also preferred injection to spraying the foliage with an iron salt solution because of the limited duration of the effect of spraying. They recommended painting pruning cuts with a concentrated iron salt solution (Rassiguier's method) for small trees, but for all others they preferred the solid " injection " method. Both methods caused damage, but this was minimized by using ferric potassium tartrate, iron pyrophosphate or ferric ammonium oxalate, iron salts which they found to be less toxic than a number of others tried. Like Chabrolin (1924), they found that in peach trees the exudation of gum into the injection hole rendered injection, either by the solid or liquid method, inapplicable commercially. Further recent French work will be mentioned later (p. 16).

Italian work

Early Italian work on plant injection was restricted to attempts to control insect attack by introducing insecticides into the host. Perosino (1899) was the first to use potassium cyanide powder for this purpose. This, when injected into a bush of *Euonymus chinese* (*sic*), seemed to cause the scale insect *Chionaspis euonymi* infesting it to be easily blown off by the wind. According to Barbero (1899), Perosino obtained definite results of a similar kind on apple trees and on vines infested with phylloxera. The latter apparently, (Perosino 1899), were injected in the autumn in order that the potassium cyanide might be carried " by the descending sap " down to the roots and so to the phylloxera parasites feeding on them. Barbero also stated that, following Perosino, similar positive results were obtained by Soave and Martinotti as well as by De Alessi and Silvestri. These results were called in question by Berlese—sitting on a Commission—his only justification being, apparently, the negative results obtained by himself and Francheschini ; but the final report of this Commission has not been traced.

About the same time, Berlese (1899, 1901) published the results of his own work. He, unlike Perosino, employed solutions. These he injected under negligible external pressure through tubing fixed directly over the ends of freshly cut root stumps, apparently in ignorance of Goff's description of the same method published in 1897, which will be referred to later. A few of Berlese's injections with tobacco extracts seem to have controlled attacks of aphides without harming the host plant ; but his results were not consistent. The only other Italian work that has been traced is that of Dezeani (1913) who used injection for purely physiological experiments. He proved that potassium cyanide solution, when injected with a syringe into plants, decomposed rapidly, and he tried, but without success, to determine its fate in the plant as a means of discovering the functions of cyanides that occur naturally in plants.

German work

It is curious that Sachs' treatment of chlorosis by injection with iron salts as early as 1880 does not seem to have been followed up to any great extent in Germany.

The work of Roth (1896) has already been mentioned (p. 10). He injected a tree with a complete nutrient solution but did not state the result. He visualized as possible advantages of the method, that trees could be supplied with exactly what they needed, including their special requirements for blossom formation, and that soil deficiencies both of these nutrients and of water could be made good more economically by injection than by watering because when applied to the soil, both are largely lost by passage to the lower layers. However, he does not appear to have followed up these ideas.

In the other German paper published during the period, Jesenko (1911) described how, by injecting $0 \cdot 1$ and $0 \cdot 01\%$ ether and 1% alcohol solutions into the cut ends of branches under one atmosphere pressure, he had shortened the rest period in *Robinia pseudacacia*. In Austria, in the same year, a paper was published by Weber (1911) who described experiments in which the unfolding of dormant buds was hastened by injecting water through a hollow needle into their bases.

Müller, in 1926, published a monograph entitled : " Die innere Therapie der Pflanzen ", in which he reviewed the literature and described his own numerous experiments. These were directed mainly to the control of insect and fungus diseases of plants by injection. He discussed at length the fact that the safety and utility of the process must depend on finding substances which are harmless to the host when injected in much larger doses than those necessary in the ordinary way to control the disease. He reported that injections of pyridine and aluminium sulphate both caused woolly aphis to leave the twigs of apple trees ; but for neither was the ratio of the " dosis tolerata " to the " dosis curativa " sufficiently high to make the discovery of practical value.

American work

American work started quite logically with the injection of water. Goff (1897) in the spring months of 1894-5-6, injected recently transplanted trees with distilled water through rubber tubing fixed over the end of a freshly cut root stump and connected to a reservoir held slightly higher than the top of the tree. Nearly every tree so treated opened its buds, many beginning within two days. He made the interesting observation that cut shoots injected under a $5\frac{1}{2}$ ft. head of water absorbed one and one-half times as much water as others totally immersed until no more was absorbed.

In 1902, Bolley (1903, 1904, 1906) started work in North Dakota, a summary of which he published in 1904 and 1906, promising a bulletin which, apparently, was never published. His method was a slight improvement on that of Roth. The hole was " at once filled with water to exclude air ". " The economic purpose " of this work, in his own words, including italics, was, " (1) if trees can be directly fed, aside from the natural source, then we can learn *what* to feed, and *how* and *when* to do it." Thus, he seems to have been the first to visualize the possibility of diagnosing a tree's nutritional requirements by injection. He proceeds, " (2) Trees are subject to two sorts of disease, (*a*) simple physiological derangement due to faulty nutrition, and (*b*) parasitic (animal and plant) " ; and he hoped by injection " to correct faulty . . . nutrition, and perhaps, so medicate the food supply as to relieve or guard the plant against parasitic attacks." In his 1906 paper, Bolley stated his belief that individual unhealthy trees could be saved by injection, and he mentioned using solutions of formaldehyde ($\frac{1}{2}$ to 2 parts per thousand), copper sulphate and ferrous sulphate, which hastened recovery of apple trees from sun scald and checked development of *Exoascus*. He concluded by saying : " plum and apple trees when fed have produced more and better fruit, larger growth and sturdier foliage, than checked trees. The chief difficulty in the way of this work becoming practical seems to be that each tree appears to be a law unto itself."

In 1914, Sanford (1914) stated that following the injection in February of a twelve-year-old Spanish broom plant, 4 inches in trunk diameter, suffering from cottony cushion scale (*Icerya purchasi*) with enough solid potassium cyanide to fill a hole $\frac{3}{8}''$ diameter and $3''$ deep (except for the plug) the scale was controlled in a few days and the plant became very vigorous. An old decrepit peach tree treated in a similar manner became more vigorous and bore a crop of peaches which tests showed were not poisonous to a chicken, a rabbit, or to Sanford himself. A year later these trees, and a similarly treated orange tree, still showed no serious damage resulting from the treatment (Sanford 1915). In the same year Shattuck (1915) reported that he had been using the injection method in his forestry work for about twelve years, against boring and girdling insects, and it had saved the lives of thousands of trees ; but he did not state how much potassium cyanide he used or the time of the year when it was injected. In the meantime Surface (1914) reported that a commercial firm of " tree doctors " was doing an extensive business in the Eastern States " vaccinating " trees by inserting capsules containing potassium cyanide, potassium chlorate and iron sulphate, into incisions made under the bark, claiming

for the treatment markedly increased vigour and immunity from all parasites, both insect and fungus, and " taking thousands of dollars from the confiding public ". Large numbers of trees throughout Pennsylvania were killed and injured, and Surface, who examined hundreds of the treated trees, appears to have been unable to obtain proof of any good effect from the treatment, either as regards vigour or disease. This report was followed by others by Flint (1915) and Wellhouse (1916) of injury and of ineffectiveness*. After this, American work on tree injection for some years seems to have been directed along more theoretical lines. Thus, Moore and Ruggles (1915) studied the movement of potassium cyanide by cutting down injected trees and applying chemical tests, and they concluded that the distribution was too localized to be of use against wood boring insects. Elliott (1917), in more detailed work, noted that the cyanide disappeared entirely within two days and he failed to detect any movement of the salt from one annual ring to another. Rankin (1917) injected lithium nitrate solution into trees by a modification of Shevyrev's method and studied its distribution by sectioning the tree and examining the pieces spectroscopically.

Two papers by Rumbold (1920a and b) contain the best review of the relevant literature up to that date. She and her helpers made slight modifications in Shevyrev's methods and applied them to a large number of chestnut trees preliminary to attempting to control chestnut blight (*Endothia*) ; but the results were inconclusive.

The cure of chlorosis by injection methods, to which a large proportion of the important Russian and other work was directed, does not appear to have been attempted in America until comparatively recent years. Hendrickson (1925), as a result of his work, could not recommend injection for its control on commercial lines. Lipman and Gordon, however, reported in 1925 that they had cured 300 badly chlorotic trees by injecting them with ferrous sulphate solution by a modification of Roth's method. A little later Bennett (1927, 1931), after trying both liquid and powder methods for this purpose, worked out practical details for applying the latter to fruit trees of various sizes. In his 1931 paper he states that " A total of about 75,000 pear trees have been successfully treated by the dry-salt method, mostly by the growers themselves." A little later Wallace (1935), in England, applied Bennett's method successfully to apples.

While Bennett's work was in progress, Thomas and Haas (1928) concluded from their own experiments with solutions on orange trees that, in view of the two facts, (1) that the cure was only temporary (2 years) and (2) that the tissues were damaged and possibly laid open to fungus attack, further work was necessary before the liquid method could be recommended as a commercially satisfactory cure for chlorosis in orange trees. Burke (1932), on the other hand, working on apple trees obtained promising results by injection with ferrous sulphate solution and also by driving iron nails into the trunk in such a way as to reach the wood and be sunk beneath the bark, taking care not to injure the latter during the operation.

Allen (1932) changed the colour of hydrangea blooms from pink to blue by injecting this shrub with solutions of aluminium salts, in this way solving a problem of some interest both from the economic and the scientific points of view. He made an oblique cut in the stem upwards for some distance in such a way that the thin slip or " tongue " thus produced could be bent aside and immersed in the solution without interrupting unduly the supply of sap from the roots.

Scherer (1927) and Jacobs (1928) both reported apparent cures of fungus disease without damage to the host following the injection with thymol in solution.

* At the present time the commercial exploitation of the method is restricted by law in Washington State. The writer is indebted to Mr. R. W. Marsh for the following reference : United States Department of Agriculture Insecticide and Fungicide Board Service and Regulatory Announcements, No. 52, November 1925, p. 1,224. Washington State : " It shall be unlawful for any person, firm, or corporation to sell, offer for sale or to supply to trees or plants by boring holes or otherwise for compensation any material as a horticultural insecticide or fungicide which relies for its effectiveness on being transferred throughout the trees or plant by the sap thereof without having demonstrated to the satisfaction of the State insecticide and fungicide board the effectiveness thereof and without furnishing the purchaser thereof a printed statement describing the material in the same manner as listed above for other insecticides and fungicides sold in closed packages. Session laws of Washington, 1923, ch. 37, p. 84."

There was a smaller wave of commercial exploitation of plant injection in England at about the same time as in America and, here, too, unfortunate results brought plant injection in general into bad repute.

During the last decade, injection methods have been increasingly used for the study of mineral nutrition in fruit trees. Lipman and Gordon (1925) published the results of injecting twenty-four ten-year-old pear trees with eleven nutrient solutions. The most striking effect was that produced by magnesium, whether used as nitrate or as monohydrogen phosphate. The foliage of four trees injected with these salts assumed a deep green colour a few weeks after treatment. In 1932, Collison, Harlan and Sweeney published a paper entitled : " Direct Tree injection in the study of tree nutrition problems ", in which, as the title suggests, attention was concentrated on the value of the method for research purposes in tree physiology and nutrition. They pointed out, independently of the present writer (Roach 1931, see later), that the soil often reacts chemically with substances applied to it and these may never reach the roots of the trees, or become changed during passage through the soil before reaching them. Injection methods hold out the possibility of avoiding these disturbing effects of the soil and consequently of testing the direct effects of the actual substances on the tree. These workers attempted to inject individual branches of a tree, using both Roth's and a branch stump method (see p. 40). They noted that with the latter method the branch cut off must be about equal in size to the one it is desired to permeate with the solution. They analysed parts of the injected trees to test the effect of the substances injected on the chemical composition of the tree, and they pointed out how natural variation in such composition between one branch and another of the same tree complicates experimental work on injection.

Chandler, Hoagland and Hibbard (1933) cured "little-leaf" or "rosette" of fruit trees by injecting them with zinc sulphate, and Demaree, Fowler and Crane (1934) cured pecan "rosette" in a similar manner.

English work

Brooks and his co-workers used injection methods both in the first stage of an attempt to cure plum trees suffering from silver leaf disease (*Stereum purpureum*), and for a purely physiological purpose. He and Bailey (1919) injected plum trees by Goff's method with solutions of dyes and disinfectants, and the larger number of recoveries amongst the injected trees as compared with untreated controls suggested to them that the fungus had been killed by some of the injected substances. Brooks and Storey (1923) determined the toxicities of a number of fungicides towards the fungus grown on an artificial medium. The most toxic of these, 8-hydroxyquinoline potassium sulphate (known also as ortho-oxyquinoline potassium sulphate, and commercially as superol or chinosol), has been used successfully by Wormald and the writer in preliminary unpublished experiments on the control of this disease in plums. Quite recently Fron (1937 a and b), whose earlier work has already been noted, induced a darker green foliage colour, and an increased vigour and resistance to diseases—in carnations to *Fusarium Dianthi* and in elms to *Ceratostomella Ulmi*—by injecting the attacked hosts with 0·05% solution of the neutral sulphate of the same organic compound, known commercially as cryptonol or sunsol. This was done experimentally either by inserting into the base of the stem a hypodermic needle connected by rubber tubing to a burette containing solution or by placing cuttings or freshly lifted small trees with the cut ends of their roots in it. The latter method has been used successfully on a commercial scale for carnations ; and the results so far obtained with elms encourage the hope that this tree may be saved from threatened extinction by the disease. Dr. Dufrenoy (1937) has informed the writer that he has obtained equally encouraging results with elms by using a modification of the earlier injection method employed by Fron (see p. 11). Dr. Dufrenoy has found that absorption is much more rapid through holes made within a foot of ground level than through those made higher up the stem, and he has kindly given permission for this interesting unpublished fact to be mentioned here (Dufrenoy 1937).

Reverting to the investigations of Brooks and his co-workers, the injection method was used by him and Moore (1926) and later by him and Brenchley (1929, 1931) to prove that aqueous extracts prepared from cultures of *Stereum purpureum* on artificial media, when injected into plum trees produced silvering of the foliage and other symptoms of the disease ; and they determined some of the properties of the substance or substances causing these symptoms.

The present writer has been working on plant injection for some years and published preliminary notes on his results in 1931 and 1933 and also a paper in 1934 entitled : " Injection

for the diagnosis and cure of physiological disorders of fruit trees." More than half of this paper was devoted to the principles underlying the control of the distribution of injected liquids. It was necessary fully to understand these before it was found possible to cause at will the uniform permeation of all the branches when a whole tree is injected, or to prevent the liquid from travelling beyond the branch or shoot concerned when only a part of a tree is to be injected for diagnostic purposes. These and other preliminary notes and papers will be referred to in the next part of this paper.

Injection work on mineral deficiency diseases

Of late years various injection methods have been used as a first, or an early, step in finding cures for a number of deficiency diseases, and the investigations concerned with this have been carried out along essentially similar lines in practically all countries. The American work on zinc has already been mentioned (p. 15). Anderssen (1932), in South Africa, cured experimentally on a few leaves a chlorosis of deciduous fruit trees by injecting the leaves with copper sulphate solution. His method is an interesting one: "Test tubes filled with a solution of $Cu.SO_4$ of 0·3 p.p.m.Cu were fixed to trees with strips of adhesive tape and a chlorotic leaf was bent so as to dip into the solution in each tube. . . . After two weeks the particular leaves which had been immersed in the $Cu.SO_4$ solution had turned perfectly green whereas the rest of the leaves on the same twig were still chlorotic . . . " This point is vividly demonstrated by his plate. Further, he states that "Occasionally the whole twig may turn green."

Storey and Leach (1933) in East Africa cured experimentally a chlorosis of the tea bush by injecting it with solutions of sulphates. Leach's method (Storey 1938), which will be mentioned again later, (see p. 40) resembled that of Collison, Harlan and Sweeney (1932 see p. 40) for branches, but was on a smaller scale. Atkinson (1935), in New Zealand, McLarty (1936) and Young and Bailey (1936) in Canada, Jamalainen (1936) in Finland, cured "cork" troubles in apples by injecting trees with boron compounds. The practical value of some of these discoveries will be discussed later.

Injection methods used in physiological work

Finally, injection methods have been used with success for purely physiological purposes. Moreau and Vinet (1932) more than doubled the number of grapes set by injecting glucose into a vine just prior to bud break. Their method was a new one. They wrapped the glucose in filter paper and placed it in a test tube the end of which was drawn out and pushed firmly into a cork. This was pressed into a hole bored into thick healthy tissue in the trunk or branch, the joint being made good with paraffin wax. The sap rose into the tube and impregnated and dissolved the contents.

Iyer, Siddappa and Subrahmanyan (1934) injected plants with certain solutions of organic origin by means of a hypodermic needle attached to a reservoir containing the liquid, and they observed marked effects on growth and sexual reproduction.

Oinoue (1935), by injecting both glucose and asparagin into grape vines by Rassiguier's method, varied both the total amounts of carbohydrate and nitrogen and the carbon to nitrogen ratio. In consequence he was able to study the effect of all three of these factors on the setting of the fruit. Other examples will be given in the next part of this paper.

The purpose of the foregoing outline of the history of plant injection has been to show the general trend of past work; the large number and variety of papers published makes a more detailed review of their contents impossible for present purposes. An effort has been made to make the list of references as complete as possible, but the papers are scattered in so many journals that some omissions may unwittingly have occurred, and the writer would be grateful to be notified of them. A number of papers published while the present investigations were being carried out will be considered further in the final discussion, because they illustrate how the methods now to be described may be applied to practical problems.

Scope of the present paper.

The remainder of this paper will be devoted to an account of the methods developed for injecting particular parts of standing trees and plants, varying in size from a single interveinal

area of a leaf to a whole main branch, each with a different liquid, and also for injecting whole trees. Brief mention will be made also of the types of problem for which each method of injection is best suited. In general, the most delicate methods in which parts of leaves are injected and compared with contiguous untreated or differently treated parts of the same leaf, make possible the diagnosis in a few days of mineral deficiency when this results in a change in leaf colour or growth rate. When the effects on vegetative growth, development of disease, or crop, are to be studied, the injection of a number of separate branches of suitable size on a single tree often enables comparisons to be made conveniently and accurately. Whole trees may be injected for experimental purposes, and in special circumstances their injection on a commercial scale is warranted.

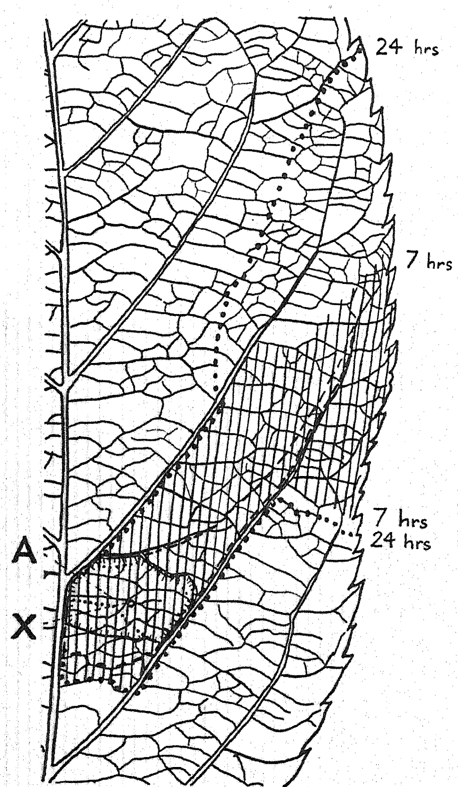
For these purposes an accurate knowledge of the distribution of the substances after injection is essential. For example, in injection for diagnostic purposes, when a number of branches or twigs or smaller parts of the same tree are treated, each with a different liquid, it is necessary to know, on the one hand, how much of each branch or leaf will become completely permeated, and, on the other, the point beyond which none of the liquid will pass. When whole trees are injected it is desirable that all branches should become uniformly permeated.

Material

The experiments have been in progress during the last seven years and the material used has varied from strawberry plants and nursery stock to fully grown plum, apple and other trees. A whole plantation of partially derelict plum trees, which was felled while the experiments were in progress, provided valuable material not often available for such purposes ; and a number of healthy, nearly full-grown apple trees, which became available, were of even greater value. The work has hitherto been confined mainly to trees, bushes and plants cultivated for fruit production, and little attention has yet been paid to the attractive problems likely to arise when the methods are extended to other plants, differing in leaf venation, branching and other morphological characters.

METHODS OF INJECTION

Many different methods of carrying out injections are possible, and they are most conveniently discussed and explained in relation to the size of the part of the plant to be treated. All methods may be employed for diagnostic purposes, each having its own range of usefulness; but only those affecting the whole tree, and to a less extent those designed for treating main branches, are likely to be of use for commercial purposes. The chief methods of injection evolved and employed up to the present will now be described, beginning with those for use on a small scale and most suitable for rapid diagnosis, and concluding with those which affect the whole plant.



Limit of spread at end of $\frac{1}{2}$ hr ....
" " " 1 hr
" " " 4 hrs - - - -
" " " 24 hrs ....
Spread at end of 7 hrs shown by hatching

FIG. 1.

The numbered lines mark the limits of permeation of a dye solution injected through an incision X in an apple leaf after varying lengths of time. The numbers refer to hours. A veinlet A was punctured by the incision.

1. Interveinal leaf injection

A PRELIMINARY EXPERIMENT

As is well-known, the loss of water from the leaf surface by transpiration results in the water in the conducting tissues being in a state of tension, and if these tissues are opened at any point under water the water is drawn in. This fact is readily demonstrated by pushing, barely through the blade of a leaf still on the tree, the wet capillary tip of a glass tube containing a suitable dye in aqueous solution. In this way the ruptured cells immediately become connected by a film of liquid with the dye solution in the tube. The movement of the dye may then be watched. Its progress and the final distribution of the dye vary according to the venation of the leaf and the position of the rupture. The results of such an experiment on an apple leaf, with reticulate venation, are shown in text fig. 1. The point of insertion of the capillary tube, shown at X, was equidistant from two neighbouring secondary veins and the midrib, and a veinlet A, connecting the midrib with a secondary vein, was perforated. The leaf and injection tube remained in contact at this point and the dye solution (0·5% patent blue) travelled in both directions along this veinlet until it reached the midrib and the lower of the two secondary veins. At the same time it spread more slowly upwards and downwards in the tissues adjacent to the veinlet, but did not cross the midrib or either of the secondary veins. Half an hour after the puncture was made the limits of spread of the dye were as indicated by the light dotted line (see key) and about 5 minutes later it had reached the limits which in the figure mark 1 hour's spread, and are shown thus . Although the colour of the area permeated deepened, the

dye did not progress further upwards for more than half an hour; it was "held up" all this time along the line of another veinlet stretching from one of the secondary veins to the other; but at the end of this time, i.e. one hour after the start, the dye began to travel along the lower secondary vein, and later it spread slowly along it toward the midrib. The roughly triangular area enclosed by the interrupted line (see key) was that occupied by the dye four hours after the start. Comparatively little movement had taken place in the direction of the midrib and none across it or across the two secondary veins. The dye had still not crossed the whole of the veinlet just mentioned. Seven hours after the start (see hatched area), the dye had crossed the upper end of the lower of the two secondary veins and had spread to the margin of the leaf; it had also spread along more than half the length of the upper of the two secondary veins, but had not crossed it or the midrib. Even after twenty-four hours (see heavy dotted line) the movement of the dye was limited by the midrib and the two secondary veins except at the upper ends of the latter, where the dye had spread across them. The much slower progress of the dye downwards may be followed in the figure. Most of the above facts are in harmony with what is already well-known. The dye solution was sucked in under the liquid tension existing in the vascular system of the leaf due to the transpiration of water from the leaf surface. The solution travelled comparatively freely along the elongated elements of the veinlets, its flow along them towards the leaf margin being limited by the high resistance offered by the large surface area of the fine vessels through which it travelled, and by the constant loss of water by evaporation at the leaf surface. The flow towards the main vein was further limited by the normal flow of fluid in the opposite direction, and the dye solution, therefore, spread further towards the leaf margin than towards the main vein. Its more rapid movement along than across the veinlets is explained by their structure. Each consists of a bundle of elongated tubes lying side by side, the tubes being divided at intervals by cross walls or septa pierced by pits, through which liquid may pass. These tubes have also a smaller number of pits in their side walls, through which liquid can pass from one tube to another, but movement from tube to tube laterally is not nearly so easy as that along the length of the tubes. Further, sap was already being drawn along them before the injection started, consequently the injected liquid, in order to travel laterally, would have to move across, not stationary canals, but rapidly flowing streams. In a secondary vein the tubes in continuation of many veinlets lie side by side, and consequently offer a still greater resistance to flow across it; in fact, the larger secondary veins and the midrib of the apple leaf are hardly ever crossed by liquids injected as in the above experiment, except at their upper ends, where they become thin.

The way in which the injected liquid permeates the whole of the interveinal area between the injection point and the leaf margin before travelling into the neighbouring interveinal areas would hardly be predicted on the basis of what is known about how the veinlets become collected, and to some extent fused, to form a secondary vein, which in turn joins with the midrib. The facts are illustrated diagrammatically in text fig. 2, in which each of the lines labelled 1 to 7 represents a veinlet and the conducting tissues in continuation of it in

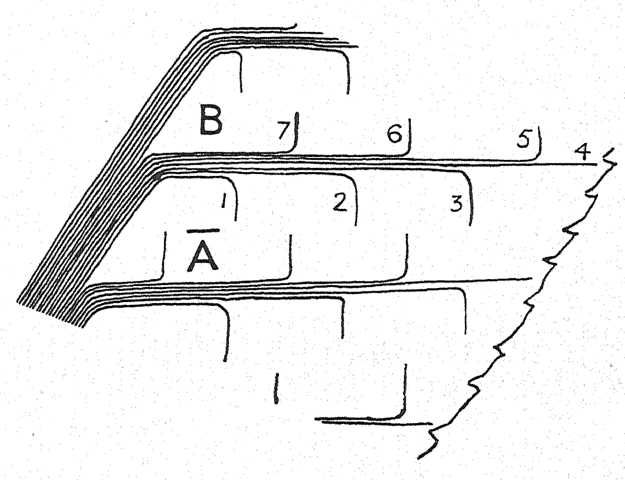


Fig. 2.

Diagrammatic representation of the arrangement of the vascular tissues in direct connexion with the veinlets, which are numbered 1—7, as deduced from the results of injections through incisions such as A. The numbered pieces of vascular tissue are, for convenience, called strands in the text.

the secondary vein and midrib. For convenience these will be called strands. Throughout this paper, for want of a better term, this word will be used to denote long thin pieces of conducting tissue, not in the narrowest botanical sense. Only three veinlets are represented on each side of each secondary vein in the figure, whereas there are more than ten times that number in the actual leaf. There are therefore more than seventy strands separating the injection point A from a point B in the next interveinal area. As the dye enters each strand it travels slowly backwards for a short distance and rapidly upwards to the end of the strand. Its direction and successive positions reached by it are therefore represented by the numbering of the strands. Strand 1 becomes permeated for its whole length, then, later, strand 2 is permeated in a similar manner. The dye may travel, apparently, round the end of the secondary vein, but actually it will permeate strands 3, 4, 5, successively.

The secondary veins are represented in the figure as flat sheets of strands. Actually they are roughly circular in cross section, and the strands, therefore, are closer to each other than as drawn in the figure; in fact they are contiguous or fused. The above facts prove that the strands are "packed" in the veins in such a way that liquid can move from one to another only in the order mentioned (not, for instance, direct from strand 1 to strand 7, the corresponding strand on the other side of the vein). These facts suggest that the strands are flattened into thin strips in parallel planes at right angles to that of the leaf blade.

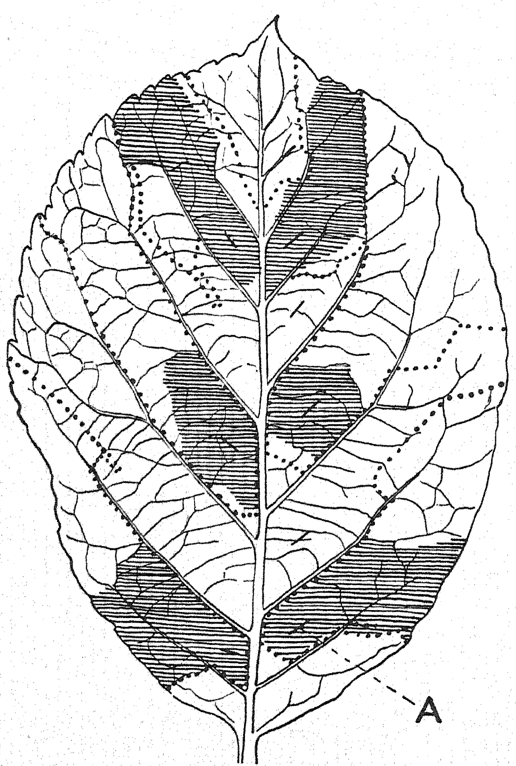


FIG. 3.

RESULT OF INTERVEINAL INJECTION OF AN APPLE LEAF.

The cross-hatched areas were permeated in 4 hours by patent blue solution injected through incisions such as the one marked A. The limits of permeation after 23 hours are marked by dotted lines. The alternate interveinal areas on the right were injected with water. This had no effect on the distribution of the dye.

THE POSITION OF THE INJECTION POINT

As will be described presently the injection is usually through a small incision in the leaf blade. Such an incision interferes least with the normal flow of sap when it is made mid-way between two neighbouring substantial veins, since it does no damage to any main or secondary vein or even to any large veinlets. If a main or secondary vein is damaged, all the tissue traversed by veinlets joining it nearer the margin may die. Wherever the incision is made the injected liquid will reach the leaf margin within about five hours, but it travels only a comparatively short distance towards the midrib. Therefore, only when the incision is made in the position selected in the experiment just described (or a little closer to the midrib), does the tissue close to the midrib become permeated. Further, the closer the incision is to the midrib, the less is the risk of the liquid crossing a secondary vein into the next interveinal area, since the secondary veins increase in thickness as they approach the main vein. This, therefore, is the best position for the incision.

DISTRIBUTION OF INJECTED SUBSTANCE

Text fig. 3 shows the distribution of patent blue solution injected through a number of incisions (e.g. A) made at such points in alternate interveinal areas on both sides of the midrib. The areas

permeated at the end of four hours are cross hatched, and the edges of those invaded in the following nineteen hours are shown by dotted lines. The remaining interveinal areas on the left hand side of the leaf were untreated, but those on the right hand side were injected with water at the same time as the above injections were started, but this had no appreciable effect on the distribution of the dye. Such injections, therefore, may be made in the apple leaf on the assumption that well-developed secondary veins are practically impassable barriers for liquids introduced at a point not more than half the distance from the midrib to the leaf margin, whether the neighbouring interveinal areas are injected or not. Even when the secondary veins become crossed there is never any uncertainty as to which injection was responsible for the resulting colour or other change, because the injected area is always continuous and never sub-divided and always has within it the incision through which the injection was made. The pair of areas lying between the base of the midrib and the first secondary vein on each side of it, as well as the terminal quarter of the leaf are less suitable for interveinal injection than the remainder of the leaf, because when the incision is made near either the tip or the base of the leaf the permeated area is less clearly demarcated than when it is made near the middle.

Duration of injection

The above and similar experiments have proved that unless injection continues for some time the permeated area does not extend far along the side of the secondary veins. The effect of the injection can be observed best when permeated and untreated areas are separated sharply by a contiguous boundary of as great a length as possible. These conditions were satisfied in the preliminary experiment described when the injection had proceeded for 7 hours; the area then invaded is watched vertically in text fig. 1. At this stage the secondary vein on each side of the incision formed a sharp boundary for rather more than half its length between permeated and normal areas. Text fig. 1 and even more so text fig. 3 show that if the injection is allowed to proceed too long (about 24 hours), the injected liquid tends to creep beyond the secondary veins, and the divisions between permeated and uninvaded areas cease to be so definite as when the secondary veins themselves constitute the boundaries. The optimum duration of injection varies somewhat from one type of leaf to another and according to the meteorological conditions; consequently, to obtain the sharpest contrasts, preliminary experiments should be carried out with dyes. With leaves of apple, pear, strawberry and Shasta daisy, however, a duration of from 7 to 12 hours has been found satisfactory.

Methods suitable for use in field

The glass tube drawn out to a capillary tip used in the preliminary experiment is quite convenient for single injections in the laboratory, but is useless for experiments in the open or when several injections have to be done on a single leaf. Two more convenient types of apparatus will be described, the first suitable for apple or larger leaves and the second for smaller leaves, for use in field or orchard.

Method I. The first method is illustrated diagrammatically in text fig. 4, A, B, C, D. The liquid, held in a reservoir, is drawn up a filter paper "wick" which passes through an incision made in the leaf. The "lips" of the incision clip the wick, which in turn supports the reservoir. A film of liquid connects the cut tissues with the wet filter paper. The tissues absorb liquid from the film and liquid travels up the filter paper to make good the loss.

The reservoir consists of a thin-walled glass tube 1·5 mm. in diameter, and about 5 cm. long. (Tubes supplied by British Drug Houses for their Capillator Sets are of suitable diameter and wall thickness.) The narrower end of a strip of a toughened filter paper (Whatman No. 54), 1·5 mm. wide at one end and tapering to 1·0 mm. in 10 cm., is drawn through the glass tube until the wider end just fits the tube. The narrower end is cut off flush with the end of the glass tube and the wider end is cut off about 0·3 cm. above the other end. The surface of paraffin wax, kept molten at 100° C., is just touched with the lower end of the glass tube, so that the wax rises about 1 mm. in the tube to form a tiny plug, which fills the end of the tube and grips the end of the filter paper strip. The tip of the protruding filter paper is dipped momentarily into Durofix solution diluted with 1½ volumes each of ether and alcohol or in a solution of celluloid

of suitable consistency so that the top mm. is permeated by the solution. On drying, this treated portion of the paper remains tough even after being moistened. An all-glass hypodermic syringe (without the needle) is filled with the liquid. A piece of glass tubing drawn out to a capillary is connected to it with a short piece of rubber tubing; the capillary is inserted to the bottom of the reservoir tube, which is then filled with the liquid, the capillary tube being withdrawn during the operation. The operation may also be carried out with a glass tube drawn out to a capillary, but the above method is much easier.

A definite incision is made with a scalpel such as that illustrated in text fig. 4 C, D, which is constructed by breaking off (with a pair of parallel jaw pliers) a conveniently shaped piece from a new safety razor blade, inserting its base into the split end of a large-size wooden match stalk, and strengthening the union with Durofix.

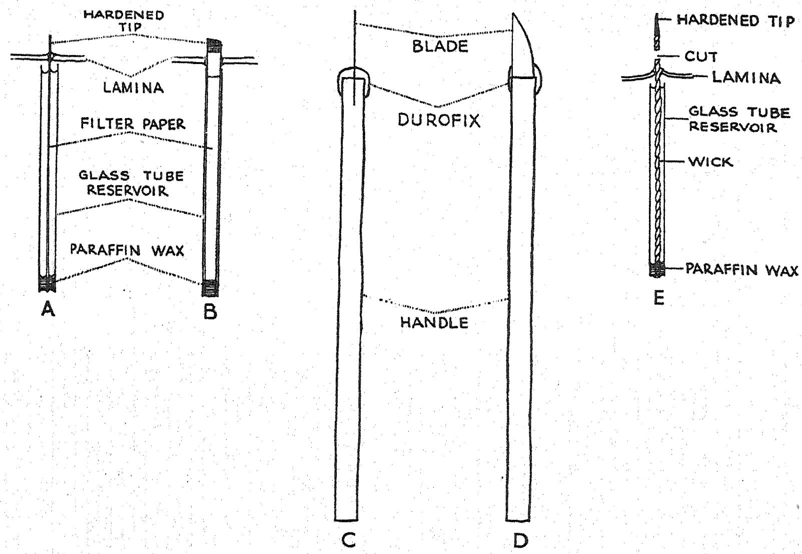


FIG. 4.

Apparatus for interveinal leaf injection. A and B are two views of the same apparatus, B being at right angles to A. C and D Scalpel for interveinal injection in two views. D being at right angles to C. E Apparatus for interveinal injection of small leaves.

Manipulation. Since mature leaves rarely respond to injection, experiments are carried out with young ones. Such fragile material is easily damaged unless some such procedure as the following is adopted. The middle, third and fourth fingers of the two hands are placed tip to tip behind the leaf, which is held between the thumb and forefinger of the left hand. With the scalpel, held between the thumb and forefinger of the right hand, an incision is made of a length equal to the width of the " wick " (i.e. 1·5 mm.) through the leaf from the under side and about equidistant from two neighbouring secondary veins and the midrib, as shown at A, etc. in text fig. 3. The hardened end of the protruding piece of filter paper, held in pointed forceps, is pushed through the incision, and finally its tip is pulled until the top of the reservoir nearly touches the leaf. If the operation has been carried out properly the edges of the incision hold the paper sufficiently firmly to support the reservoir, even in a moderate breeze. The tubes tend to drop off when all the liquid has been absorbed and the wick is no longer connected to the leaf with a liquid film, which supports the weight of the tube.

The method just described is the most satisfactory one for carrying out the operation in that the minimum damage is done to the leaf, the incision being perfectly clean; but some of the writer's colleagues prefer to push the hardened end of the filter paper strip through the leaf blade without previously making any incision. This may be done quite easily, but there is a

tendency for the tissue to be torn rather than cut and the operation carried out in this way is never as neat as when a definite incision is made with the scalpel.

Method II. The second method is simpler than the first and is applicable to smaller leaves. In it the filter paper " wick " is replaced by a piece of darning cotton which serves the same purpose. The method is illustrated in text fig. 4 E. A 5 cm. length of darning cotton is broken off. The end is pointed by rolling between the finger and thumb, as if for threading a needle, and dipped in diluted Durofix solution as before. This makes the end stiff. The thread is then passed through a piece of glass tubing similar to that used for method I, so that its untreated end is flush with one end of the tube. This end is dipped momentarily in high melting-point paraffin wax kept molten at 100° C. so as to plug it and hold the end of the thread. The reservoir is filled in the same way as in method I. The hardened end of the thread is pushed through the leaf blade at the desired point and carefully pulled through until the top of the reservoir nearly touches the lower surface of the leaf blade ; finally the excess of thread is cut off, as shown in the figure.

Time of detection of response to injection

As already pointed out, the conditions under which the treated and untreated areas of leaf are compared are nearly ideal in that they are separated only by a narrow secondary vein and in that the comparison can be made along both sides of the treated area ; in these circumstances a slight colour change is readily detected which would be quite impossible by other methods, as for example, when comparison is made between whole plants, the individual leaves of which usually show considerable variation in colour. The quickest response so far observed was given by an injection of 0·05% iron citrate solution into a chlorotic apple leaf (see p. 24), a slight but quite definite green tint being observed 3 days later. The green colour increased in intensity for a further seven days. In other experiments, seven days have elapsed before a response has become definitely discernible. Experience so far obtained suggests that the full response is given in ten days.

Effect of age of leaf on response to injection

The experience obtained with this second method which so far is somewhat limited suggests that a half-grown leaf is the best. Responses have been obtained with immature leaves when mature ones have given no visible response. Further, when such immature leaves have been injected there has sometimes been observed, in addition to an improvement in colour, greater growth of the permeated as compared with the untreated areas ; this has naturally resulted in a puckering of the leaf.

Substances injected

Because of the striking colour change brought about by the injection of iron compounds into one type of chlorotic leaf most attention has so far been paid to this element ; but experiments are being carried out with others including the principal nutrient ones.

Hill in work to be published in detail shortly, has diagnosed by these methods, or slight modifications of them (see below), nitrogen deficiency in broad bean, and phosphorus and boron deficiency in tomato plants.

Plate I, fig. 1, reproduced by the kindness of Mr. H. Hill, shows the result of injecting an interveinal area of a broad bean leaflet with urea. The effect was only just detectable with certainty by the naked eye, but photography made the effect much more apparent.

Kinds of leaves

The methods described have been applied successfully to apple, pear, and plum leaves, and strawberry leaflets, the venation of all of which is reticulate-pinnate. Hill has used them successfully on the broad bean (Pl. I, fig. 1) and on mature tomato leaflets ; but when applied to young tomato leaflets (Pl. I, fig. 2) the liquid does not remain confined within the area delimited by the two secondary veins, one on each side of the incision. The writer has had the same experience with peach leaves.

Presumably the number of veinlets united in the short secondary veins of these long narrow leaflets or leaves are insufficient to present a serious barrier to the movement of injected liquid across them. The conditions, in fact, are similar to those in the apple leaf when the incision is made midway between the main vein and the leaf margin or even nearer the leaf margin, when, as has been shown, the liquid crosses the secondary veins. In working with young leaflets such as those of the tomato, a half leaflet is the smallest practicable injection unit. The incision is made near the base of the leaflet on one side of the midrib. The injected liquid then remains on one side of the midrib and the tissues on the other side of it may be used for comparison. In the peach a half leaf is the smallest practicable unit.

Application of interveinal method

The problem for which the method was actually evolved will serve as an example of its practical application. Small apple trees grown in a nutrient solution developed severe chlorosis. In the absence of any definite indication as to which element was lacking the effects of a number of nutrient salts, both alone and in combination, were tried by this method. In three days one interveinal area injected with iron salt was slightly greener than the surrounding tissues; and on the fourth day this and two other areas injected with an iron salt combined with other nutrient salts were definitely darker green than untreated areas, thus proving in four days that the trouble was due to iron deficiency.

A week after the injection the permeated areas had assumed a healthy glossy dark green colour, and the fact that these areas were raised in puckers demonstrated that they had grown faster than the surrounding untreated tissues.

Because the treated areas were so small these tests caused the small trees no appreciable damage. For the same reason it was possible to prove that the gradation in colour shown by the leaves on each shoot of these trees was due to iron deficiency which became more severe as each succeeding leaf unfolded, and, a point of greater importance, the early stages of iron deficiency were demonstrated. Each of the first three or four leaves was of a lighter green than its older neighbour, the green was of a "metallic" quality, and the leaves appeared to lack "finish"; the leaves succeeding these showed the well-known symptoms of iron deficiency chlorosis, namely a yellowing around the leaf margin, especially towards the tip, tending to spread between the veins towards the midrib in severe cases. In very severe cases even the veins themselves were of a yellow colour quite free from green. The early stages of this trouble characterized merely by a light unhealthy type of green had not previously been associated with iron deficiency. The fact was proved by injecting leaves in all stages and getting an improved green colour except in the darkest green ones; and the importance of even this early stage of the trouble was suggested by the increased growth of the injected areas. (Roach 1937 and Roach and Levy 1937.) This trouble appears to be widespread throughout Kent and elsewhere.

Statistical Significance of Interveinal Leaf Method

This must obviously depend on how often there occur in nature single interveinal areas, of healthier appearance than those on each side of them such as could be mistaken for the effects of artificial interveinal injection. Careful search has been made for the past two seasons, but among the many thousands of leaves examined in orchard and garden only a single instance of such an occurrence has been observed. The odds against the selection of that particular area for an injection are, therefore, more than 1,000 to 1; and if the experiment is done in duplicate the odds become 1,000,000 to 1, a degree of certainty rarely achieved in biological experiments. Moreover, the above calculation takes no account of the fact that an actual change is observed (e.g. of colour), and that this takes place during a predictable and relatively short period of time. This fact still further enhances the reliability of the method.

The above considerations apply whenever a positive result is obtained by this method, no matter how small the change in the appearance of the area may be. But when, for example, the colour of the area changes, as a result of an injection, from yellow to a much darker green than that of any leaf on the plant, the diagnosis approaches complete certainty.

II. Leaf tip Injection

(a) Simple leaves

This method consists in cutting off the tip of a leaf or leaflet at right angles to the midrib and at once submerging the cut edge below the liquid. The method is particularly suited to long narrow leaves, such as those of the peach, but it may be used for almost any type. A convenient procedure is illustrated in text fig. 5. The liquid is drawn in through the cut edge of the leaf under the tension set up by transpiration and, as it travels along the veins and veinlets, it is continually used by the leaf tissues to make good transpiration losses. Its flow is of course impeded by the high frictional resistance offered by the large surface of the fine spaces through which it flows, and the extent of the injection is also limited by the flow of sap in the opposite direction.

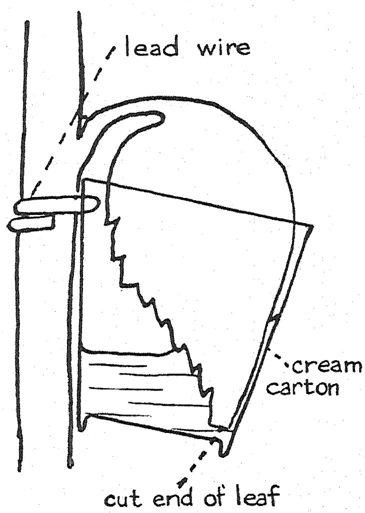


Fig. 5.
Leaf tip injection.

The greater the distance of the cut from the leaf tip the larger is the number of veins and veinlets severed, and consequently the greater is the distance from the cut edge that may become permeated by the liquid, therefore this statement will have to be modified when leaves with parallel venation are injected. It is necessary to make preliminary injections with dye solutions with each kind of leaf to decide how much to cut off to obtain any desired degree of permeation, but the following figures will give some idea of results likely to be obtained with apple or pear leaves. If a portion equal in length to one-tenth that of the midrib is removed about half the leaf will become permeated; if equal to one-fifth the whole remaining part of the leaf will become permeated; if equal to more than one-fifth not only the rest of the cut leaf itself but part of all the basal halves of the two leaves, situated respectively above and below the treated one on the twig, will also become permeated. It is best, therefore, to aim at permeating no more than three-quarters of the leaf, so that there shall be no risk of the injected liquid travelling into other leaves, which would limit the choice of an untreated leaf, necessary as a control. A consideration of the results of leaf stalk (petiole) injection in the next section will make this matter clearer.

With apple and pear leaves enough liquid is usually absorbed in about ten hours, and the response to the injection of an iron salt by this method is usually apparent in a week or ten days (Roach 1936). The slight increase in greenness which is detectable by the interveinal method of injection previously described within three days, when comparing areas on each side of a secondary vein or the midrib, is usually quite imperceptible when comparing an injected whole (tipless) leaf with an untreated control. Hence, although the leaf tip decapitation method is easier to carry out, the interveinal method is preferable when rapidity of response is essential. Nevertheless, the leaf tip method may have advantages for certain physiological experiments.

(b) Compound leaves

(i) Each leaflet of a compound leaf may be injected separately by cutting off its tip and immersing the cut edge of the leaflet in the liquid to be injected. Thus, one lateral leaflet of a strawberry leaf may be so treated and the other left untouched for comparison. (ii) If the cut is made so as to remove one-quarter, or little more, of a strawberry leaflet, the injected liquid travels into the nearer side of the adjoining one. Thus, after a little practice with dyes, it is possible to inject through the cut tip of a lateral strawberry leaflet in such a way as to affect the whole of that leaflet and almost exactly half of the terminal one (text fig. 6) in which the treated and untreated tissues will be separated sharply by the main vein for most of its

length. If the terminal leaflet is injected in a similar way, it and the nearer half of both the lateral leaflets become permeated (text fig. 7).

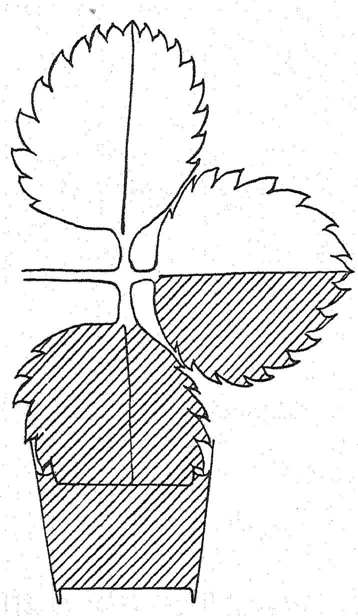


FIG. 6.

Injection of strawberry leaf through the tip of a lateral leaflet. Cross-hatched areas are permeated.

STATISTICAL SIGNIFICANCE OF LEAF TIP METHOD

The degree of certainty with which a symptom of improved health in an injected leaf can be attributed to the injection must depend on the proportion which the number of leaves of equally healthy appearance bears to the total number of comparable untreated leaves on the tree. If the appearance or size of the injected leaf is not beyond the normal range of variation of the leaves of the tree, the reliability of a diagnosis can be calculated quite simply by the ordinary laws of chance ; but should the appearance or size of the leaf definitely exceed this range, the certainty of the diagnosis is greatly enhanced. An example will make this point clear: If the leaf after injection becomes the most healthy in appearance of the (say) 100 leaves on a tree it may be argued that the odds against this one leaf being merely by chance the healthiest are 100 to 1 ; and this statement would give a measure of the certainty of the improved health being the result of the injection. When, however, the appearance of the injected leaf is definitely outside the range of variation of the leaves on the tree, e.g. when all the leaves on a tree are chlorotic and the injected one takes on a healthy green colour after being injected with a solution of an iron salt (Roach 1936), the improved health is practically certain to be due to the substance injected, and the reliability of the diagnosis is almost absolute.

III. Anderssen's leaf immersion method

Anderssen's own description of this interesting method has already been given earlier in this paper (p. 16) but the writer so far has had practically no experience of it. A comparison of the results given by it and by the preceding method would be of obvious interest, and this will be made as soon as opportunity offers.

Its mode of action is easily understood. The immersion of the entire leaf in solution would stop its own transpiration and consequently the flow of sap from the stem into the leaf. It is well known that liquid placed in contact with leaves enters the stomata. Here, as soon as it makes contact with the liquid system of the plant, it will be drawn into the conducting tissues by the tension in the system ; and when it has discharged this tension, it will be drawn into neighbouring conducting tissues and will travel thence into those leaves which have conducting tissues in direct connexion with them. The extent of this permeation of

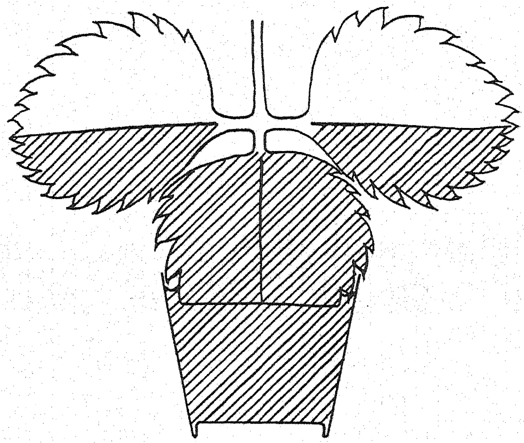


FIG. 7.

Injection of a strawberry leaf through the top of the terminal leaflet. Cross-hatched areas are permeated.

neighbouring unimmersed leaves obtained by Anderssen suggests that the high resistance to the movement of liquid into the uninjured stomata is largely compensated by the very large number of them distributed over the area of leaf surface in contact with the liquid.

IV. Leaf stalk injection

(a) Apple

The fact already mentioned, that when more than one-fifth of the leaf is removed in preparation for leaf injection, the injected liquid travels into other leaves, suggested that useful types of distribution of the injected liquid might be obtained by removing still more of the leaf. Descriptions of a few experiments in which the whole of the blade was removed and the remaining leaf stalk was injected with dyes will indicate some of the kinds of distribution obtainable.

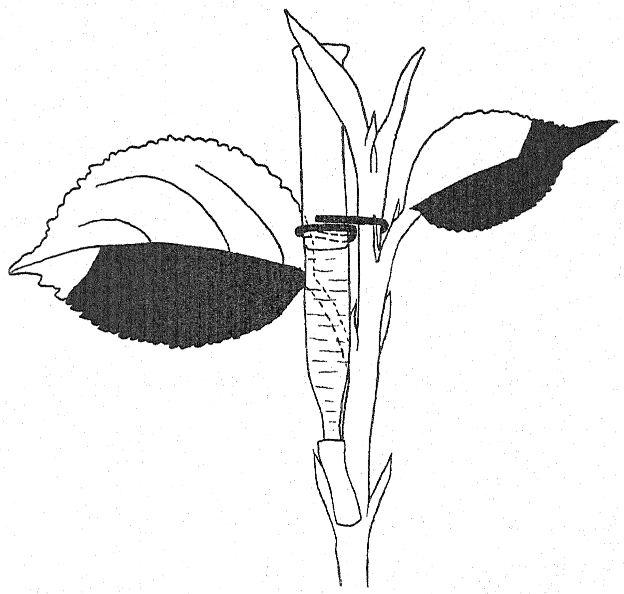


FIG. 8.
Leaf stalk injection. The liquid is held in a glass tube which is attached to the leaf stalk stump with rubber tubing. The permeated areas are represented black.

A leaf towards the base of a current year's apple shoot was cut off so as to leave a short length of stalk attached to the twig. To this was connected the drawn-out end of a small test tube by means of bicycle valve tubing, the upper end of the tube being fixed to the stem with lead wire or rubber surgical tape (text fig. 8). The tube was filled by a hypodermic syringe with 0·5% aqueous solution of acid fuchsin, care being taken to wet the cut end of the leaf stalk and to expel all air from the narrow rubber tubing. There was a perceptible reddening of parts of certain of the neighbouring leaves in a few minutes, and the distribution of the dye could be mapped after about fifteen minutes. The intensity of this reddening continued to increase, but there was no further spread of the dye at the end of twenty-four hours.

The resulting distribution of the dye is shown diagrammatically in text fig. 9 which is drawn as if the leaves had been detached from the shoot and arranged at points on a phyllotaxis diagram corresponding with their positions on the stem. The results are also expressed in a different form in Table I.

The fraction of the area of the leaf permeated by the dye decreased as its angular distance from the injected leafstalk (O) increased; the leaf directly above it (No. 8), was permeated throughout its whole area, whereas the fourth leaf up the spiral, which is on exactly the opposite side of the stem to the injected leaf stalk, was not reached by the dye at all. The corresponding leaf (4) down the spiral was also quite free of dye. Leaves 3, (3), and 5 (i.e. the third leaf up, the third leaf down and the fifth leaf up the spiral) which were distant only 1/8 circumference from the injected leafstalk were permeated except for a small area at the base of the blade on the side remote from the injection point. Leaves 2, 10, 6, (2), distant 2/8, were coloured over slightly more than half their area ; and leaves 1, 9, 7, (1), distant 3/8, were permeated over slightly less than half their area. The diagram makes the fact clear that the permeated part of a leaf extended as an individual area from the base of the blade on the side nearer to the injection point, up that side of the midrib, nearly to the leaf tip ; in fact, no other part of the leaf became permeated unless this proximal basal area was permeated. Conversely, a small basal area on the opposite side of the leaf never became permeated unless the remainder of the leaf was also

TABLE 1

		Fraction of leaf blade permeated.	" Angular " distance from injected leaf stalk.
Above injected leaf stalk.	10	Slightly more than half.	2/8
	9	Slightly less than half.	3/8
	8	Whole.	0/0
	7	Slightly less than half.	3/8
	6	Slightly more than half.	2/8
	5	Nearly whole.	1/8
	4	None.	4/8
	3	Nearly whole.	1/8
	2	Slightly more than half.	2/8
	1	Slightly less than half.	3/8
Injected leaf stalk.	0		
Below injected leaf stalk.	1	Slightly less than half.	3/8
	2	Slightly more than half.	2/8
	3	Nearly whole.	1/8
	4	None.	4/8
	5	Nearly whole.	1/8

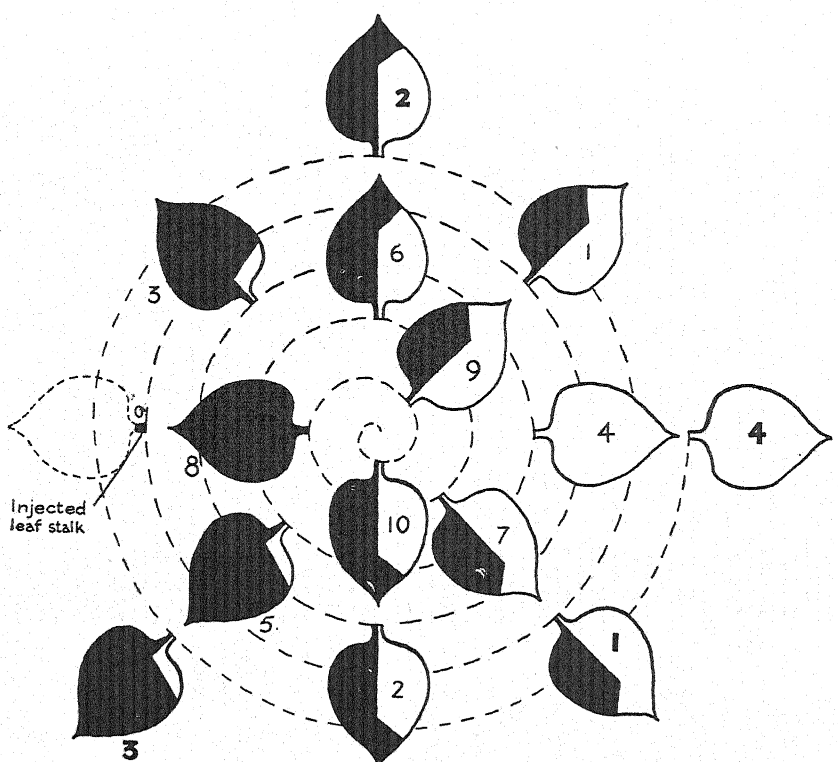


FIG. 9.

Phyllotaxis and leaf stalk injection pattern of the apple shoot. 0 is the injected leaf stalk. Permeated areas are represented black.

permeated. Other experiments showed that the actual fractions of the leaf areas permeated could be decreased at will below that shown in text fig. 9 by making the cut nearer the leaf tip. As already stated (p. 25) if the length of the tip cut off is not more than one-fifth that of the midrib, no injected liquid reaches other leaves. Hence, as the length of tip removed increases from one-fifth to the whole blade together with most of the leafstalk, so the permeated areas of other leaves on the shoot increase from zero up to those shown in fig. 9. As the amount removed from the injected leaf increases, so, for example in leaf 3, the permeated area increases. At first a small basal area on the proximal side of the midrib is affected, then this increases up its proximal side, rounds the tip of the leaf and extends down the distal side until only a small area, such as that shown in the figure is left unaffected.

In eight experiments (four with 0·5% acid fuchsin and four with 0·5% patent blue) in which the cut was made half way along the leafstalk of a leaf near the middle of the current year's shoot, the resulting distributions were practically the same.

In these experiments the liquid travelled rather less freely downwards than upwards, actually reaching the tenth leaf upwards but only the fifth one downwards. In other experiments in which leaf stalks nearer the top of the shoot were selected, so that the upward movement was limited by the number of leaves above the injected leaf stalk, the downward movement tended to increase rather than decrease; and when the stalks of leaves which had just become fully expanded were used movement downwards was actually greater than that upwards. Closely similar results were obtained with shoots which had finished extension growth. The distribution of dye in leaves which were still folded followed the same rules.

These facts show that liquids may be injected into apple leafstalks by this method with considerable confidence as to what the actual type of distribution will be; and experiments with colourless liquids can be designed so that the most sensitive leaves, i.e. the youngest, become injected in such a way as to be of the most use for diagnosis. Perhaps the most generally useful arrangement is to inject the stalk of the youngest leaf that is stiff enough to hold the rubber tubing; the two or three leaves above it are usually still unfolding, and consequently are likely to respond fully to any nutrient supplied; moreover there are also two or three leaves below, any of which may not be too old to show some degree of response. In all of these leaves the basal proximal quarter becomes injected and the corresponding distal quarters remain uninjected. In the basal halves of these leaves the treated and untreated areas are divided sharply from each other by the midrib vein, and this is an almost ideal arrangement for showing any small difference in colour, rate of growth, etc., which has already proved of great use in diagnosing faulty iron metabolism.

Sixteen injections of 0·05% solutions of iron citrate and ferrous sulphate have been carried out on apple trees in the Research Station plantations (Roach and Levy 1937). The distribution of a bronzing, which resulted in a few injections from slightly excessive doses, and the increased depth of green observed later in these experiments, followed the same laws as those governing the distribution of dyes.

Sectorial injection of apple fruit by leaf stalk method

Preliminary experiments carried out by Levy with dyes have shown that if a leaf stalk injection be carried out on a spur carrying a fruit, either the whole fruit or only a single sector of it may be permeated, according to the position of the injected leaf stalk in regard to the fruit. The size of the permeated sector may be varied in a manner similar to that in which the area of permeation of leaves may be controlled.

Applications of leaf stalk method

The comparison of treated and untreated areas is nearly as sharp in this method as in the interveinal one; consequently any response to the injected substance can be detected nearly as surely and quickly by it. It is easier to carry out and is less liable to be vitiated by rain and wind, but the effect of only one single substance can be tested on a particular shoot. Following the results obtained with the interveinal method mentioned on p. 24 the leaf stalk method was used to test representative trees throughout the Research Station plantations. The injections proved

that the majority of the trees were suffering from incipient chlorosis, related to iron metabolism, (Roach and Levy 1937). Accumulating evidence suggests that this trouble is widespread throughout Kent and elsewhere and is likely to be of considerable economic importance.

Statistical significance of leaf stalk method

During the past two seasons a close watch has been kept for colour or other variation in leaves which might be mistaken for the result of leaf stalk injection. With the possible exception of an ash shoot, to be mentioned later, no contrast distributed at all like the areas of leaf surface permeated as a result of a leaf stalk injection was observed. Positive results with this method of injection may therefore be interpreted with even greater certainty than those with the inter-veinal method.

Although the leaf stalk method has hitherto been applied in practice only to the apple, it is obviously applicable to other plants. A few examples will, therefore, be given of the types of distribution to be expected from leaf stalk injections carried out on other plants; and the manner in which the type of distribution of injected liquid is influenced by the vascular anatomy of the shoot will be touched on. The leaves of some plants have a groove running down their leaf stalk, and the method described for the apple is inapplicable. These may be treated conveniently by one of the two methods illustrated in text figs. 10 and 11, which scarcely require elucidation.

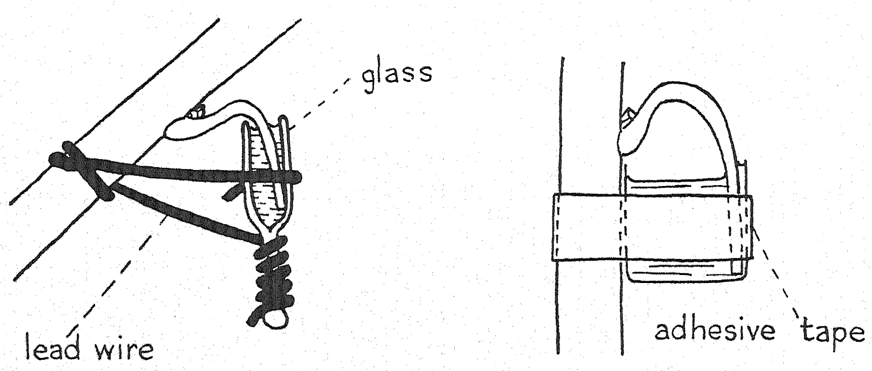


Fig. 10 and 11.
Injection through leaf stalks with a groove.

(*b*) Raspberry

The phyllotaxis of the raspberry is the same as that of the apple, namely 3/8, but the type of distribution resulting from a leaf stalk injection is not the same as may be seen by comparing text figs. 9 and 12. The more important differences seem to arise from the fact that the three main conducting strands which supply each raspberry leaf are so wide apart when they enter the base of the leaf stalk that they subtend 3/8 of the circumference of the stem, whereas the apple leaf stalk has only one main conducting strand. In text fig. 13 the central conducting strand of the injected leaf stalk is labelled 0, the left hand one 0L, and the right hand one 0R; and the left hand, central and right hand strands of the leaf stalk above and below the injected one are labelled 1L, 1, 1R; **1L, 1, 1R**, respectively, and so on.

The leaf just below **1** and the one just above 1, the injected leaf stalk, both have their two lateral leaflets on the side nearer to the injected leaf stalk permeated by dye. The reason for this can be seen in fig. 13, in which is indicated the distribution of the conducting strands as ascertained by stripping off the bark. The left hand conducting strand of the leaf below **1L** is immediately below the right hand bundle of the injected leaf stalk 0R. The strand in continuation of 0R, after travelling down the stem on meeting **1L**, divides into two and these pass on each side of 1L and of its continuation down the stem. Reference to text fig. 12 makes it clear that dye must have moved from the strands in direct connection with the injected leaf stalk

(shown dead black) into those in continuation of 1L down the stem (shaded). The left hand strand of the injected leaf stalk 0L is immediately below the right hand one 1R of the leaf just above the injected leaf stalk. Strand 1R in descending the stem meets 0L, divides into two and these pass on each side of it. Injected liquid, therefore, is drawn, by strands 1R, which are in direct connexion with a transpiring leaf, from strand 0L which is in direct connection with the injected liquid.

The permeation of these leaves resembles that of the corresponding ones on a similarly treated apple shoot in that, in both, the area at the base of each leaf on the side of the midrib nearer to the injection point becomes permeated, whereas the corresponding area on the opposite side of the midrib remains unaffected ; but the actual paths are different in the two. The permeation of the next leaf up the stem differs markedly from that in the apple. In that, as has been shown already, rather more than half this leaf becomes permeated, as shown in text fig. 9

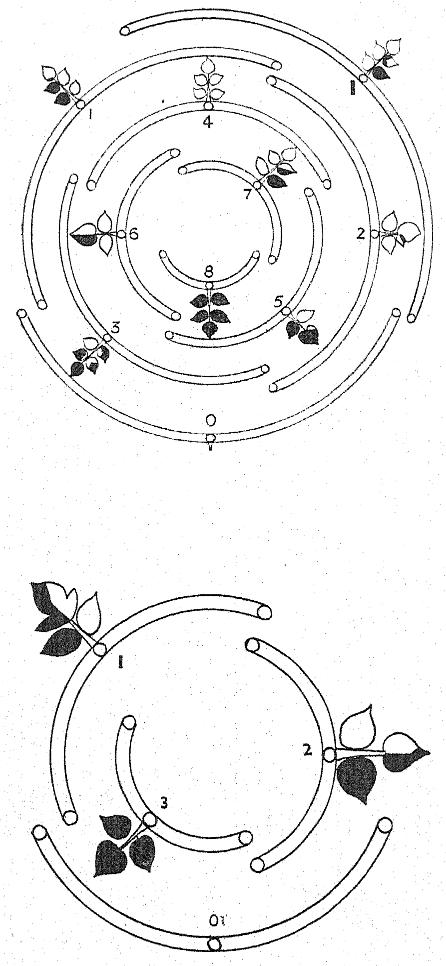


FIG. 12 and 14.

Phyllotaxis and leaf stalk injection patterns of mature and young raspberry shoots, respectively. 0 is the injected leaf stalk. Permeated areas are represented black.

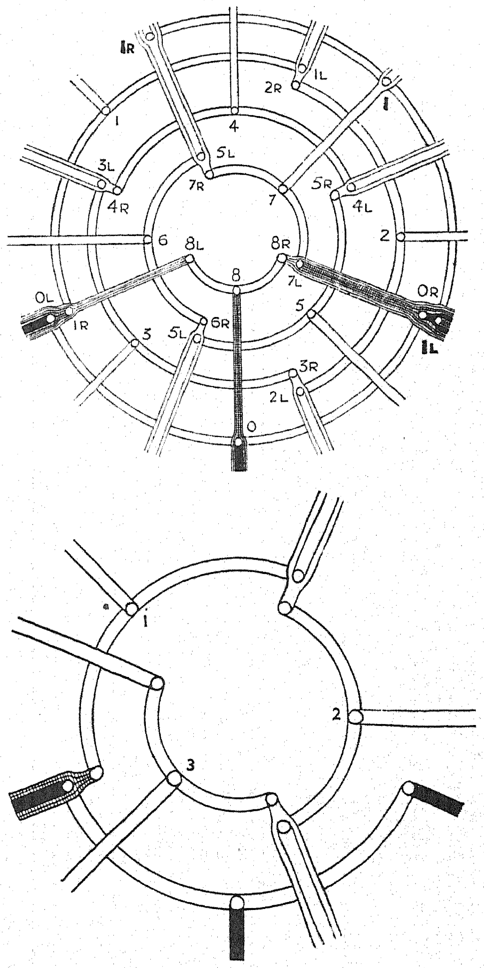


FIG. 13 and 15.

Arrangement of vascular strands in mature and young raspberry shoots. For description see text.

leaf 2, but the only part permeated in leaf 2 in the raspberry (text fig. 12) is the distal half of the second leaflet on its side nearer to the injection point. It will be seen that the right hand strand 2R of this leaf comes nowhere near any permeated strand ; the central strand 2 is separated from the injected strand 0R both by the one from 7L and by part of the one from 8R ; and strand 2L is separated from the injected strand 0 by half of strand 3R and half of strand 8. These facts are in harmony with the result that leaf 2 has only a small area permeated about half way along the midrib on the side nearer to the injection point. The actual type of permeation of the rest of the leaves may be seen in text fig. 12, and the reason for them may be traced in text fig. 13.

There is yet another difference between the type of permeation in the raspberry and that in the apple. In the latter it is the same whether an old leaf, some way down the stem or a young one near the top is injected ; but in the raspberry the results are quite different. This may be seen in text fig. 14. Leaf 1 (text fig. 14), just above the injected young leaf stalk, has become rather more completely permeated than the corresponding one above the old injected leaf stalk (leaf 1, text fig. 12), and leaves 2 and 3 above the young injected leaf stalk, have become more completely permeated than the corresponding ones above the old injected leaf stalk. The reason for this is seen in fig. 15. In it there are no strands such as 5L, 7L and 8R which, in fig. 13, separate 2L from 0R ; and such as 7 L and 8R which separate 2 from 0R. Injected liquid, therefore, is able to move much more freely into leaves 2 and 3 when a young leaf stalk is injected than when an old one is so treated.

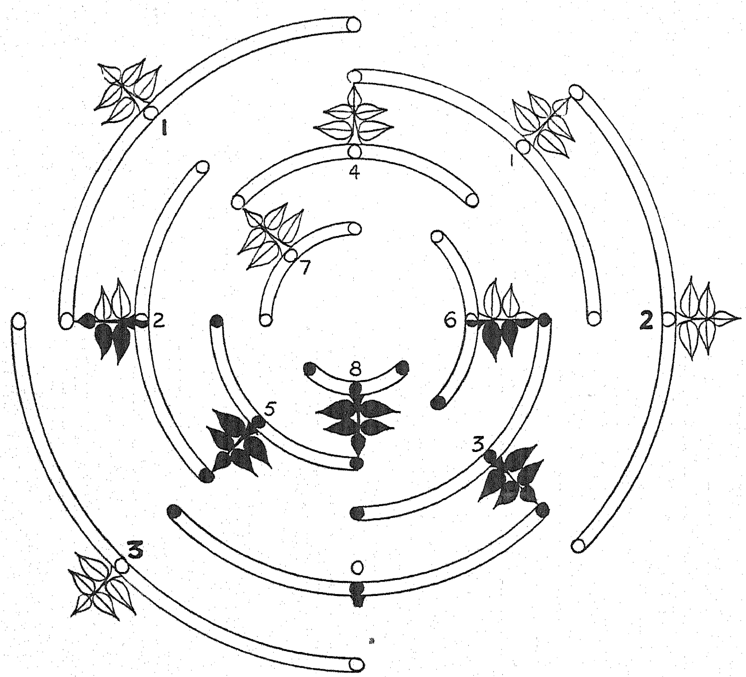


Fig. 16.

Phyllotaxis and leaf stalk injection pattern of young potato shoot. 0 is the injected leaf stalk. Permeated areas are represented black.

An injection of the type shown in text figs. 12 and 13 may be useful in physiological experiments, but for the diagnosis of mineral deficiency the stalk of a just-expanded leaf must be injected, so that young leaves able to respond to the injected substance will become permeated. When this is done, it will be seen from text fig. 14 that the first and second leaves above the injected leaf stalk become permeated, each on its own side of the main vein nearer to the injection point, and unaffected on the other. Text fig. 14 also shows that, though this type of partial permeation is not quite as satisfactory in the raspberry as in the apple, it is a useful one for diagnostic purposes.

(c) Potato

The phyllotaxis of the potato, like that of the apple and the raspberry, is 3/8. The potato leaf, like that of the raspberry, is supplied by three separate conducting strands, which are so spaced as to subtend about $\frac{1}{4}$ of the circumference of the stem. Although the three

conducting strands of the potato leaf are widely separated from each other, strands from leaves higher up the stem never pass between them. A typical result of a number of leaf stalk injections made by Hill (1938) on half-grown potato plants raised in a greenhouse are shown in text fig. 16, and the arrangement of the conducting strands in the stem is shown in text fig. 17. The injected strands are deep black and those supplying permeated leaves or parts of leaves are shaded, two depths being distinguished. The results will be seen to differ markedly from those with the apple and the raspberry.

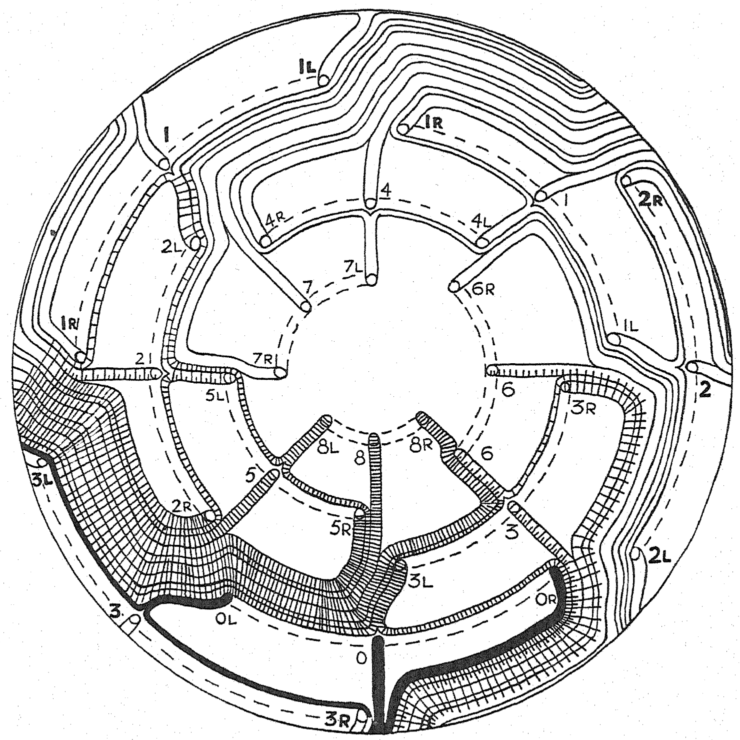


FIG. 17.
Arrangement of vascular strands in young potato shoot.

(*d*) PEAR

The phyllotaxis of the pear is 2/5, and the leaf is supplied by a single conducting strand. The types of permeation resulting from injecting an old and a young leaf stalk of a pear are shown in text figs. 18 and 19 respectively. The latter suggests that for diagnostic purposes the stalk of the highest fully expanded leaf should be injected. The lower halves of the second and third leaves above it then become permeated on the side of the midrib nearer to the injection point and not on the other.

(*e*) GOOSEBERRY

The results of preliminary experiments on the gooseberry are shown in text fig. 20, in which two rows of leaves are shown. Those in row A are from a shoot which was still growing and those in B are from a shoot which had stopped growing. There are large differences between the two shoots ; but it seems likely that further experiments will reveal how to inject so as to obtain predictable results, and so enable gooseberries to be injected by the present method for diagnostic purposes.

(*f*) RED CURRANT

The phyllotaxis of the red currant is 1/3. Growing and non-growing shoots gave the same type of distribution whereas in the gooseberry they are different. The third leaf above the injected leaf stalk was the only one permeated, but in it only one sector was permeated, as shown in text figs. 21 and 22. The figures show that the permeated sector is on the left of the leaf in a shoot with a left handed, and on the right in a right handed, spiral.

All the leaves so far considered are arranged spirally on the shoots bearing them, and in botanical terminology are said to be " alternate ". Hydrangea, coffee and hop will serve as examples of plants with so-called " opposite " leaves.

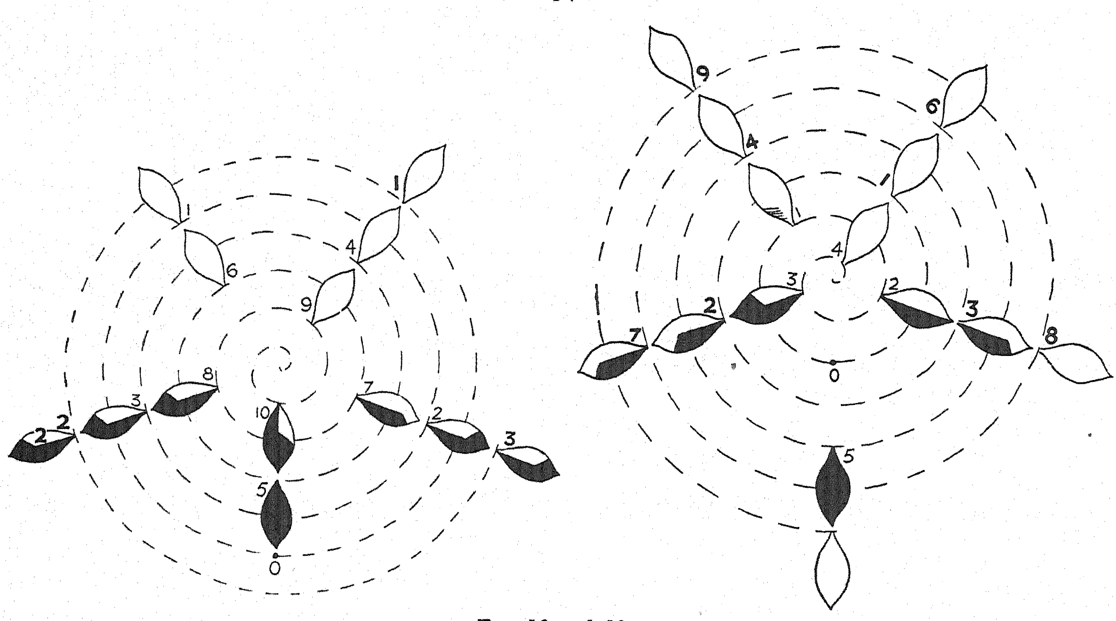


FIG. 18 and 19.

Phyllotaxis and leaf stalk injection patterns of mature and young pear shoots. 0 is the injected leaf stalk. Permeated areas are represented black.

(*g*) HYDRANGEA

Caldwell (1930b) has determined the type of distribution resulting from leaf stalk injection of hydrangea the leaves of which are opposite and decussate. His results are shown in the same manner as the rest of the figures in this paper in text fig. 23 from which it can be seen that in both leaves of the pairs next above and below the injection point the half nearer the injection point becomes permeated and the further one is unaffected. Of the next pair, both above and below, the leaf immediately above the injection point is completely permeated, and the opposite one is unaffected. The effect of treatment may, therefore, be compared in these leaves in a manner reminiscent of that obtained by Eyre and Salmon (1916) who treated one hop leaf with a fungicide and left the opposite one as a control.

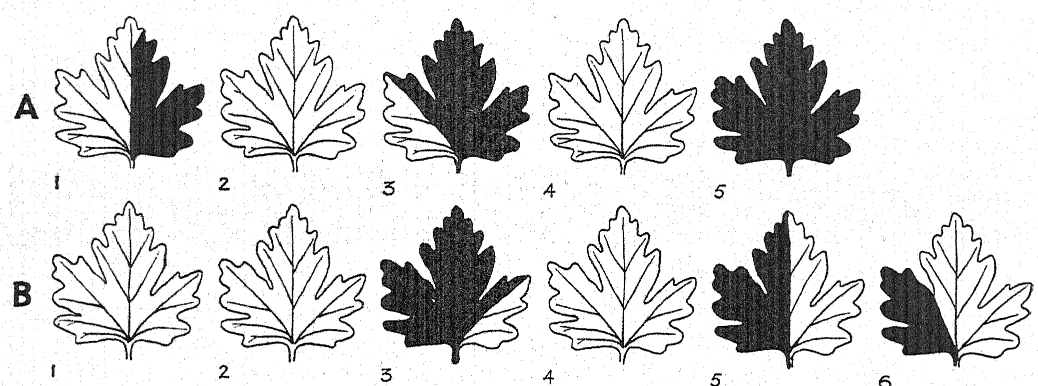


FIG. 20.

Leaf stalk injection of gooseberry shoots. A still growing. B mature.
The leaves are numbered from the injected leaf stalk (0) upwards. Permeated areas are represented black.

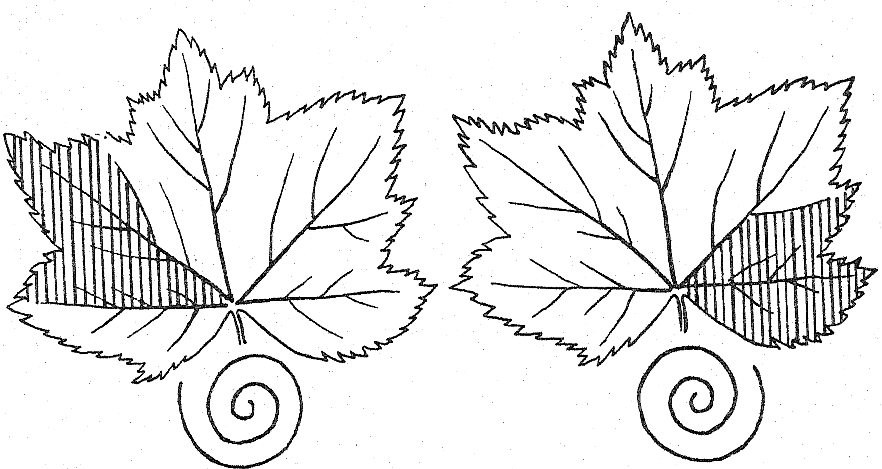


FIG. 21 and 22.
Leaf stalk injection of red currant. Third leaf above injection point. 21, a leaf from a shoot with a right-handed spiral; and 22, a leaf from a shoot with a left-handed spiral. Permeated areas cross-hatched.

(*h*) COFFEE

Case, working at East Malling on two small coffee bushes, kindly supplied by the Royal Botanic Gardens, Kew, has recently shown that the leaves of the coffee bush are sufficiently nearly opposite and decussate for the present purpose and that consequently, in the pair of leaves immediately above and below the injection point, the half of each leaf nearer the injection point is permeated and the farther one is unaffected, the midrib forming a sharp dividing line except sometimes near the tip; in the pairs of leaves next to these above and below them the leaves vertically above and below the injection point are each completely permeated and the opposite leaves are unaffected. Since his return to Kenya, Case (1938) has confirmed these conclusions on the plentiful material there available. The coffee shoot, therefore, appears to be ideal for leaf stalk injection.

The leaves of the two coffee bushes at East Malling exhibited a phenomenon first described by Goppelsroeder (1889, 1901). An hour or so after injection, the dyes began to fade, doubtless through the formation of their leuco-compounds. This, combined with the fact that the leaves were already of a healthy dark green colour, made difficult the decision as to what tissues became permeated.

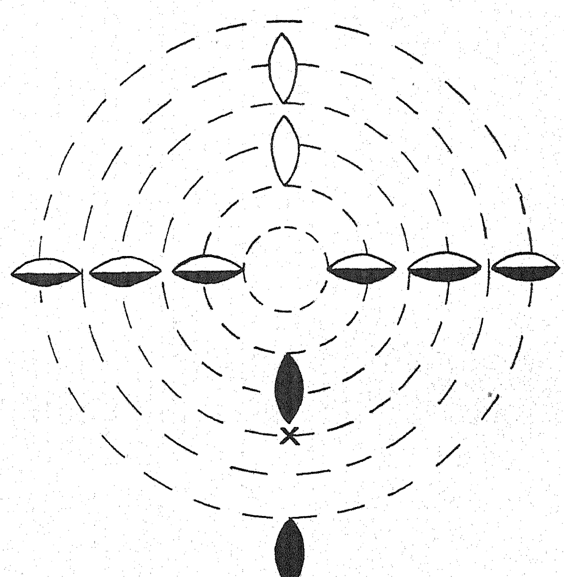


FIG. 23.
Phyllotaxis and leaf stalk injection pattern of hydrangea. Injected leaf stalk X. Permeated areas represented black.

(*i*) HOP

The hop, the leaves of which, as in hydrangea and coffee, are opposite and decussate, gave an entirely different result. This is shown in text fig. 24. Whereas in hydrangea the leaf opposite

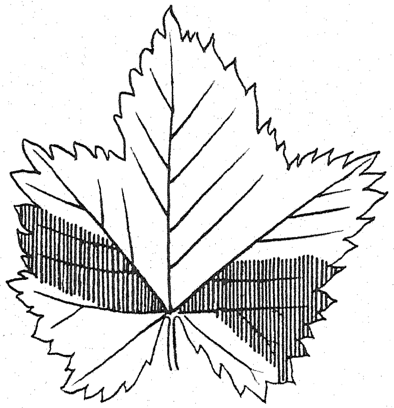


FIG. 24.
Leaf stalk injection of hop. Leaf opposite injection point. Permeated areas cross-hatched.

to the injected leaf stalk did not become permeated while those immediately above and below did, in the hop the leaves immediately above and below did not become permeated and only the leaf opposite the injected leaf stalk, and indeed only two small sectors of it, became permeated. It is hoped to carry out more detailed work on the hop shortly, one aim of which will be to see how the variation of leaf structure from the base of the plant to the top affects the type of injection pattern.

(*j*) MANGOLD

Finally the mangold will serve as an example of a plant in which the leaves arise from a crown. The result of an experiment on this plant is shown in text fig. 25. The two leaves above and below the injected leaf stalk became permeated, but each only on the side nearer to the injection point.

Further work will be necessary on all the above plants, except the apple, before leaf stalk injection work can be carried on with them for diagnostic purposes. In the meantime the preliminary experiments, here described and discussed, outline the field of work necessary and give some idea of the results likely to be obtained.

V. Shoot tip injection

In all the methods so far described injection took place through cuts made in various positions in the leaf. In the method next to be described the cut is made in the shoot.

If the tip of a shoot be cut off and the cut end by some convenient means be kept wet with liquid, the shoot becomes injected through the cut, and the material travels along the shoot and permeates one or more of its leaves. Two convenient methods of carrying out the operation are illustrated in text figs. 26 and 27, one for shoots the ends of which can be bent and one for those sufficiently stiff to support a glass tube and its contents. The cut must be made far enough from the growing point to open fully differentiated conducting tissues. How much to remove to permeate any desired number of leaves is best decided on the basis of preliminary experiments with dyes (see pp. 18 and 27.) The removal of half an inch from the tip of a shoot which has stopped growing and is fully hardened often results in three or more leaves becoming permeated; whereas to achieve the same result in a rapidly growing shoot three inches or more must sometimes be removed. The removal of less than one quarter of a current year's shoot which has stopped growing usually results in all its remaining leaves becoming permeated, and the removal of one-half results in the permeation of some of the leaves on neighbouring current year's shoots.

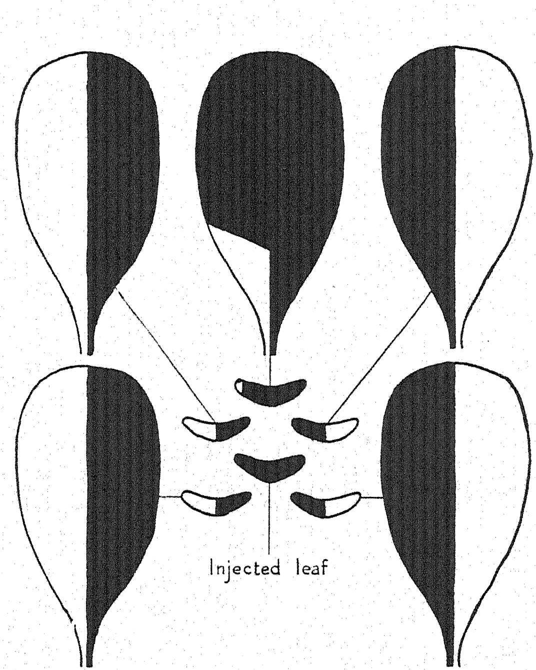


FIG. 25.
Leaf stalk injection of mangold. Arrangement of leaf bases shown in the middle. Permeated regions represented black.

Reason for localization of effects of shoot tip injection

The sap in all the conducting strands before being cut was in a state of tension as a result of the transpiration pull of the leaves. In an apple tree this state of tension exists in the strands at least down to ground level (see also pp. 47 and 56). On the strands being cut, this tension does not disappear immediately, and when the cut surface is bathed in the liquid, the liquid is drawn into the conducting strands. This occurs whether the cuts are made under water, a method used by others whose work is considered later, or in the open air in the manner adopted by the writer. The tension is reduced to zero at the open ends of the cut strands, but as the liquid flows along the very narrow spaces it is impeded by the great friction developed, and consequently the normal tension is reduced less and less as the length of conducting tissue traversed increases.

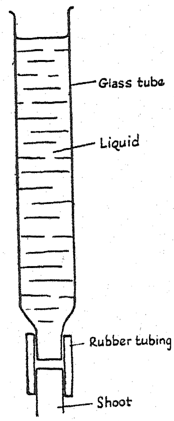


Fig. 26.
Shoot tip injection of stiff shoots.

When the first leaf is reached, these conducting strands divide and pass on each side of the strands in direct connexion with the leaf. In text fig. 28 the directions of the strands are represented by lines, those in most direct connexion with the cut end by thick continuous lines and those most remote by thin interrupted lines. These lines indicate directions only. The transpiration pull of the leaf tends to keep these strands in a state of full tension, and lying side by side with them are the permeated strands which are under only comparatively slight tension; there is, therefore, a flow of liquid from the permeated strands into those in direct connexion with the leaf, which in consequence also become permeated. As already pointed out, as the liquid invades the shoot, friction impedes its flow progressively and the tension in the liquid increases; in addition, as the shoot is descended there are intercalated strands in direct connexion with other leaves, the transpiration pull of which further increases the tension. Hence the tension increases rapidly as the distance from the cut increases, and a point is reached, sooner or later according to the number of strands cut, where equilibrium is reached. The tension conditioned by the friction impeding the downward movement of the injected liquid, added to that produced by the pull of the leaves upwards, equals that in the system as a whole at this equilibrium point. The tension decreases steadily from the leaves to the roots, in which there is sometimes a positive upward pressure. While the injection is in progress, liquid flows in opposite directions towards this equilibrium point, wherever it may be, the injected liquid downwards and the sap upwards; but none of the injected liquid can travel beyond that point as long as the permeated leaves exert their normal pull. If these leaves are damaged, however, they do not exert their full pull and the injected liquid may then be able to travel farther into the plant, the equilibrium point having shifted.

Application of shoot tip method

Three applications of this method have been described elsewhere. In the first of these it was used in June 1932 on a single apple tree to take advantage of an unusually heavy infection of powdery mildew (Roach 1934a). Ten pairs of shoots were selected so that each member of a pair was comparable with its fellow in regard to its position on the tree, vigour, severity of mildew infection, etc. One shoot of each pair was injected with water and the other with M/100 sodium thiosulphate solution. Six weeks later the leaves on seven of the thiosulphate-injected shoots were both more vigorous and decidedly less heavily infected than the corresponding water-injected

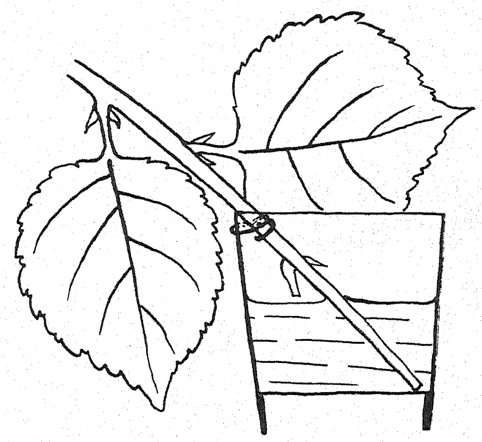


Fig. 27.
Shoot tip injection of shoots which may be bent.

shoots, although the condition of the members of the remaining three pairs had not altered. The chances of this result having been obtained by accident are less than one in a hundred (see below). Thus a valuable fact, which may eventually be turned to practical advantage was obtained with little expenditure of labour or damage to the tree.

The same method was used to diagnose the cause of a severe chlorosis in a peach tree in a greenhouse (Roach 1935b), and more recently it has been used to prove that an economically serious unhealthy condition of cherry trees in the Sittingbourne district is due to faulty iron nutrition (Roach and Levy 1937).

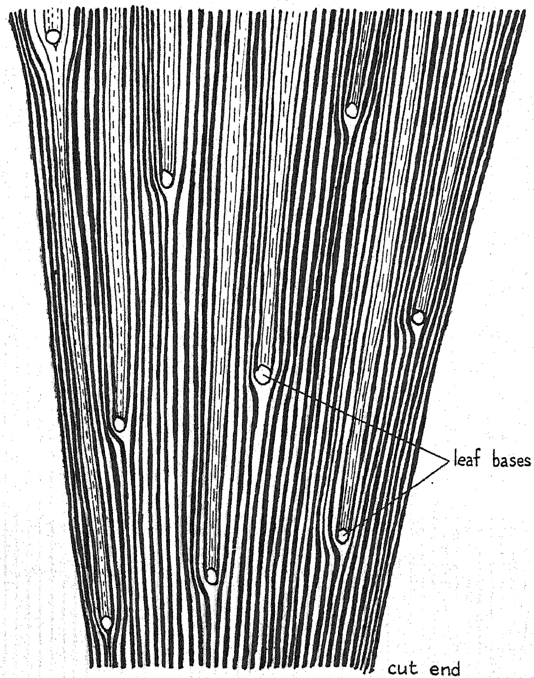


Fig. 28.

Shoot tip injection. The lines represent the direction of the conducting strands. The heavy black lines represent strands in direct connexion with those actually cut. Those most remote laterally from the cut strands are represented by dotted lines. (Drawn from a negative kindly supplied by Dr. Storey.)

Statistical significance of shoot tip method

The first example, given above, of the use of this method will illustrate how its statistical significance may be gauged. Three pairs of shoots were unaffected by the disease and consequently can be ignored. In all the remaining seven pairs of shoots the disease on the thiosulphate-injected (treated) shoot became reduced whilst that on the water-injected (untreated) one remained severe. In the first pair the one disease infection has two possible hosts and the chances of its affecting the untreated shoot are therefore 1 in 2, and the same is true of the second pair; and the chances of the untreated shoot being diseased in both the first two pairs is $\frac{1}{2}\times\frac{1}{2}$ and the chance of all seven untreated shoots being diseased are $(\frac{1}{2})^7$ or 1 in 128.

Had it been thought justifiable to consider all the shoots comparable with each other, the following argument would have been applicable. Of 10 untreated shoots 7 were diseased; the remaining 3 untreated shoots and all 10 treated shoots were free from disease. If the treatment had had no effect, the first disease infection could have fallen by chance on any one of 20 shoots, of which 10 were untreated, the chances of it falling in the untreated lot being, therefore, 10/20. The second disease infection could have fallen by chance on any one of the remaining 19 shoots, of which 9 were untreated; the chances of its falling in the untreated lot being therefore 9/19; and the chances of the first two disease infections falling by accident in untreated lot of shoots are $10/20\times9/19$; and of all 7 infections falling on untreated shoots $= 10/20\times9/19\times8/18\times7/17\times6/16\times5/15\times4/14=1$ in 646.

The same arguments would have applied had the figures referred to a colour change not outside the normal range of variation of the foliage on the branches of the tree. It is low in comparison with the results obtained by interveinal and leaf stalk injections. The reason for this difference is that the various leafy shoots on a tree commonly vary considerably in their colour and in respect to freedom from or infection by disease, whereas the kind of variation produced by interveinal and leaf stalk injections is most unlikely to arise naturally.

In the other two examples the leaves on single injected shoots changed from a yellow-green to a healthy dark green which was definitely more intense than that of the foliage on any

other shoot on the tree. Both the change in colour in a predictable period of time, and the mere occurrence of single leafy shoots of a healthy green colour on an otherwise yellow-leaved tree are so uncommon that a diagnosis based on the results of an injection is of extreme significance. (See Plate 2, frontispiece.)

VI. Experiments with larger shoots and branches. Branch tip injection.

When only about one quarter of its length was cut from the end of a current year's shoot and it was then injected, the dye completely permeated all the remaining leaves and wood of that year's growth ; on tracing it backwards, its intensity decreased continuously, until at about half way along the previous year's wood it disappeared. On entering the two-year-old wood the dye coloured the inner and the outer of the two rings about equally, but it faded out more rapidly in the inner than in the outer ring as it proceeded. When the injection was made half way along the two-year-old wood the dye disappeared in that of the previous year. When made in the middle of the three-year-old wood the dye traversed the four-year-old and disappeared in the five- or the six-year-old wood. In one branch, injected in the middle of the three-year-old wood, the dye persisted till the nine-year-old wood was reached. The dye always lasted longest in the outermost ring of wood, next longest in the adjoining inner ring, and so on. These facts are in harmony with what is known of the structure of woody stems.

When in any of these experiments the dye encountered a lateral branch of more than about half the diameter of the one into which it had been injected, it was sometimes found to ascend the wood of the branch but along its inner side only, leaving the outer side free. Thus, it sometimes happened that about one-third of the leaves on such a branch became deeply stained whilst the rest remained normal. This failure of the dye to reach many of the leaves increased as the distance of the leaves from the injection point increased.

Injection into wood more than one year old is usually best avoided, because of the "thicket" of young shoots which in response to the wounding are often caused to grow out near the cut. In practice large branches of fruit trees are occasionally shortened, as when trees are "dehorned", and if the remaining stumps of these happen to be furnished with large numbers of small shoots (fruiting spurs) these would form suitable material for branch tip injections. As will be seen later, should more than about half the length of such large branches be removed, the injected liquid will reach the main crutch. All the branches may be injected, if desired. Thus, a control one may be injected with water only, and each of the others with a different liquid without any risk of mixture. Any branches left uninjected, however, will absorb liquid from any of the others with the conducting strands of which their own are united. Further, if the soil is dry, those portions of the root system in direct connexion with individual branches will also become permeated, each with its own liquid.

If one main branch be cut off, leaving a stump which is then injected by a suitable method (to be indicated later), the liquid will enter those branches the strands of which come in contact with those of the injected branch in the crutch and trunk. This matter will be discussed more fully later. When the injected strands come into contact with those of all the other main branches, such an injection may result in a practically uniform permeation of the whole tree.

Application of Branch Tip Method

An actual application of this type of injection, carried out by the writer in co-operation with H. Wormald, will serve as an example of how it may be applied in more practical problems. It is customary to remove branches of plum trees suffering from silver leaf as soon as many of the laterals begin to die. The causative fungus (*Stereum purpureum*) is sometimes localized in the branch and is entirely removed with the branch, but sometimes it extends into the part of the trunk which supplied the branch and even into other branches. Under the latter conditions the removal of such a branch presents an opportunity for injecting a fungicide into the wood so as to surround completely any still localized fungus growth, which may remain in the wood. Moreover, if the branch is large enough, and if its actively conducting strands unite with those of all the other branches in the main stem, it may be used for injecting the whole tree with a nutrient solution in order to stimulate it to "grow away from" the fungus. Indeed, the fungicide and the nutrient may be combined in a single solution. The work in

progress on these lines is an extension of that of Brooks and Bailey (1919), who, as already stated (see p. 15), injected fungicides including 8-hydroxyquinoline potassium sulphate through root stumps cut for the purpose.

VII. Leach's shoot injection method and branch injection method of Collison, Harlan and Sweeney

In joint work with Storey (Storey and Leach 1933, Storey 1938) Leach evolved a simple and effective method for shoot injection which has already been mentioned on p. 16, and is illustrated in text fig. 29 which has been drawn from a negative kindly supplied by Storey. This suggests that these workers used shoots of approximately equal sizes for injection by immersion in a liquid, and for permeation. Experience with apple and other trees has shown that the cut shoot immersed in the liquid must be at least as large as the one to be permeated, otherwise permeation will not be complete. Further, if this condition is satisfied, the method should be applicable to shoots and branches of any size. As applied to larger branches, this method with slight modifications becomes that of Collison, Harlan, and Sweeney (1932) who, as already stated, observed that the injected and the permeated branches needed to be about equal in size. The general paths of distribution of the introduced liquid along the shoot below that used for the injection are similar to those in leaf stalk and shoot-tip injections of small branches. For larger branches distribution follows the paths described in a subsequent section, which deals with the injection of branches by a method applicable to almost any tree.

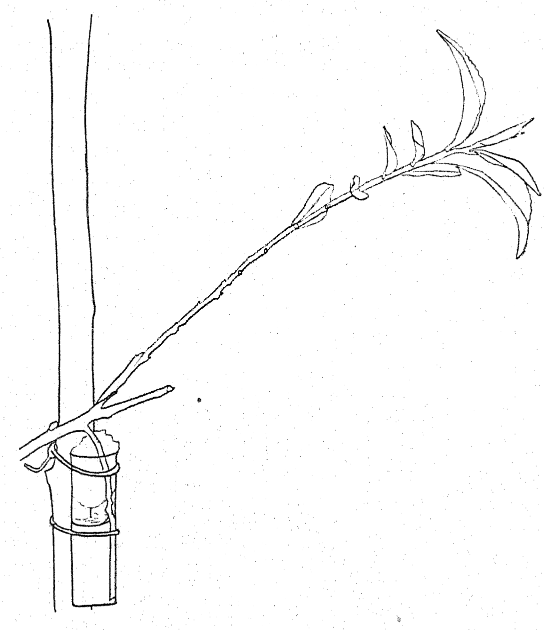


FIG. 29.
Leach's method for shoot injection.

Application of Leach's method

This method was used by Storey and Leach on small shoots of the tea bush, and enabled them to show that a chlorosis of great economic importance was due to sulphur deficiency. It has a range of applicability similar to that of the shoot-tip method and has the advantage of retaining the sensitive expanding leaves at the growing tip. Its field of use, however, is somewhat limited by the necessity of using two shoots, about equal in size, arising near the branch tip. There is no difficulty in practice in deciding which is the better method for a particular problem. In both methods account has to be taken of the stimulus given to the growth of other parts of the tree by the removal of a piece of a shoot or a branch. This removal of part or practically the whole of a shoot or branch, necessary in both methods, limits their use to small scale diagnostic injections, except occasionally when for special reasons larger branches may be removed.

Application of the method of Collison, Harlan and Sweeney

When large branches are to be injected use is generally made of a method, to be described in the next section, in which no appreciable mechanical damage is done to the tree; but when there is no objection to the removal of a considerable portion of a tree the branch method of Collison, Harlan and Sweeney may be used.

VIII. Injection of individual branches

A considerable proportion of fruit trees in certain countries are spur-pruned. As a result of this their tops consist of a varying number of main branches, usually ten or more, often very similar to one another. These lend themselves readily for injection experiments in which several branches on a single tree can be treated, each in a different way, so that a comprehensive experiment can be carried out on a single tree. Each main branch on such a tree has a considerable number of small branches arising from it at more or less uniform distances along its whole length. With such material the range of penetration following injection may be increased beyond that usually possible with the branch tip or Leach's methods. For this purpose a solution of the substance to be tested is held in a reservoir A (text fig. 30) attached to the branch B, and the solution flows thence through rubber tubing C under a few inches head of pressure into an injection hole D, drilled through a diameter of the branch, and of a bore equal to about one-eighth that of the branch or one-twenty-fourth of its girth.

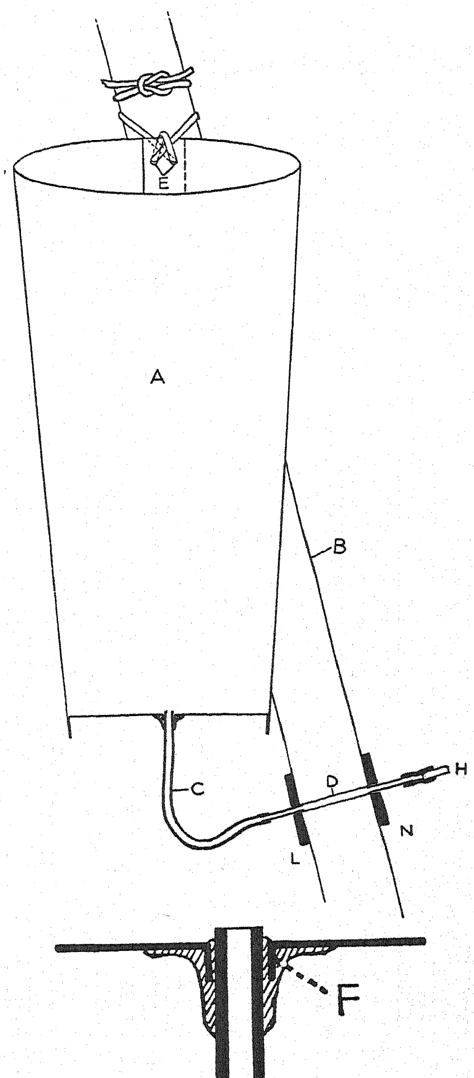


Fig. 30.

Method for injecting small branches and trees. The liquid is held in a milk carton A, attached to the branch B, and led through rubber tubing C, to the hole D which passes through a diameter of the branch. For further explanation see text

Distribution and localization of injected liquid

As is well known, the pull of the leaves on the sap in the woody tissue of the tree keeps it in a state of tension; consequently, the injecting liquid rushes up the conducting channels actually severed by the hole and so eventually into the leaves, provided, of course, that the experiment is done when the tree is in leaf and transpiration is active. These facts and those which follow are readily demonstrated by injecting a suitable dye solution (see p. 61) and later on removing the bark from the stem and so laying bare the surface of the wood. The rate of travel upwards varies but is of the order of $\frac{1}{2}$ inch per minute. The dye also travels circumferentially round each annual ring of the wood, at the rate of about $\frac{1}{20}$ inch per minute until the whole circumference of the branch at the level of the hole becomes permeated. Accordingly, soon after the injection has been started, every leaf above the hole draws on the coloured liquid; and, during the progress of the injection the flow of sap is replaced by that of injected liquid.

The injected liquid also moves down the branch at a rate which at first is about equal to that of the upward movement. When dye is injected so that the progress of the injection may be watched and the wood laid bare by carefully removing the bark, two bands of colour, each of the width of the hole, are seen to start, one from each end of it, and travel downwards. It follows the directions of faint streaks which may be seen in

the surface of the wood. The long thin pieces of wood (or strands using the word in its non-technical sense) which may be detached from the trunk also follow the same direction. This is also the direction in which the wood can be split most easily. As the dye travels round the circumference of the branch, these bands broaden until they meet, and then the whole circumference has become permeated. When the first side-branch (A in text fig. 31) below the hole is reached, the colour is seen to branch, follow the strands, and pass down on each side of it, leaving temporarily uncoloured those (left white or drawn lightly in the figure) immediately below the side-branch and in direct connexion with it. The figure is drawn looking straight at the stump A of the side-branch, which has been cut off just above the swelling, where it joins with the main stem. While injection is going on, transpiration by the leaves borne by this side-branch tended to keep the strands in direct connexion with it in a state of full tension, whereas that in the permeated strands is reduced by the inflow of injected liquid; coloured liquid, therefore, flows from the first strands into the second and a band of colour is seen to ascend the side-branch on its side nearest to the main branch. This band broadens and, if the diameter of the side-branch is not more than one-quarter that of the main branch, it will become completely permeated. The same fact is illustrated in text fig. 32 which represents a short length of spur-pruned branch in which the strands in direct connexion with side-branches are left white. The whole stem at the level of the injection point is permeated and is drawn black. As in text fig. 31, when the first branch A is reached the injected strands divide and flow on either side of the side-branch. The same thing happens as each succeeding side-branch is reached, so that when the second side-branch B (text fig. 32) is passed, the originally undivided cylinder of injected strands becomes divided into two; when the third is reached into three, when the tenth is reached into ten parts, and so on. The flow of the injected liquid is impeded by the high resistance offered by the walls of the fine tubes along which it travels, and it is being drawn on by more and more absorbing side-branches as the main branch is descended. Further, the normal sap will flow upwards as soon as a certain suction is established. It is obvious, therefore, that a point must soon be reached beyond

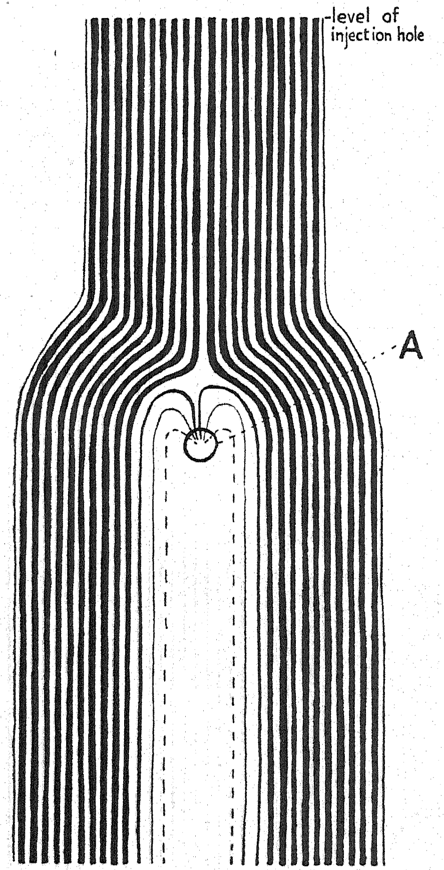


FIG. 31.
For description, see text.

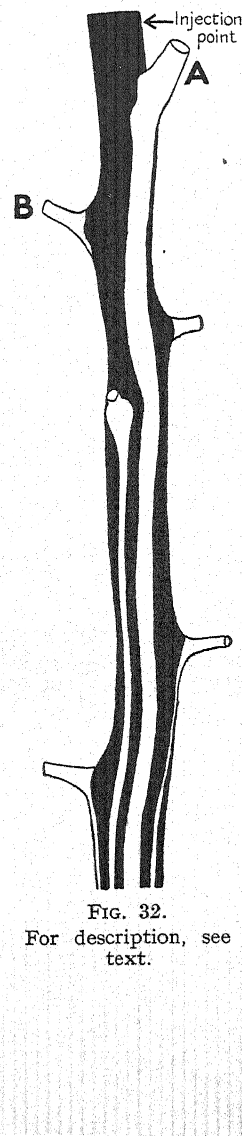


FIG. 32.
For description, see text.

which the injected liquid is unable to travel. As might be expected, this point is reached more quickly in a spur-pruned main branch which has on it many side-branches than in a main branch which has few side-branches or none at all.

Position of hole

When the branch is well furnished with small side-branches, such as with fruiting spurs on a spur-pruned tree (text fig. 33), the injected liquid does not travel more than one-third as far downwards as it does upwards, in which latter direction, as already stated, it reaches the tip. In such material, therefore, the hole is bored at a point, such as X, one-quarter the distance from the desired limit of penetration, the crutch, to the the branch tip. When base of a branch is not well furnished with side-branches the hole must be made higher up; and when a branch has no side-branches on the lower half of its stem it may

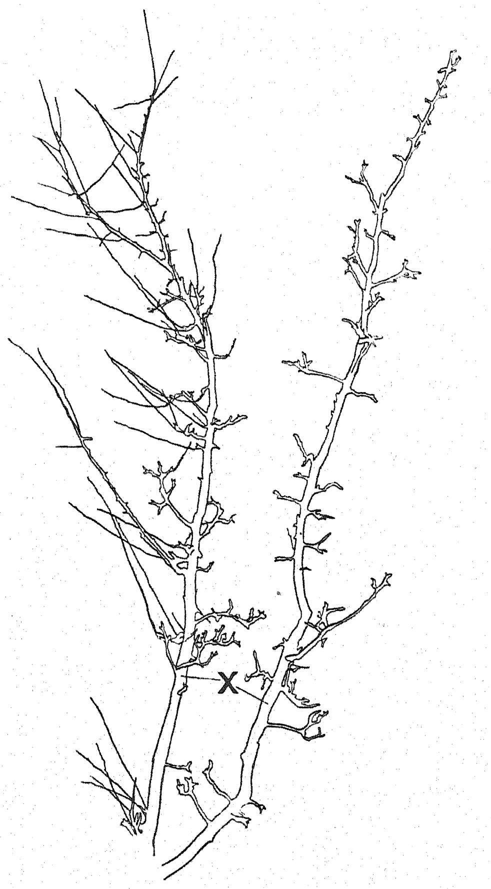


Fig. 33.

Injection of branches with many side branches. X suitable injection points, right hand branch untreated, left hand branch injected with a complete nutrient (0·5% urea+0·5% dipotassium hydrogen phosphate).

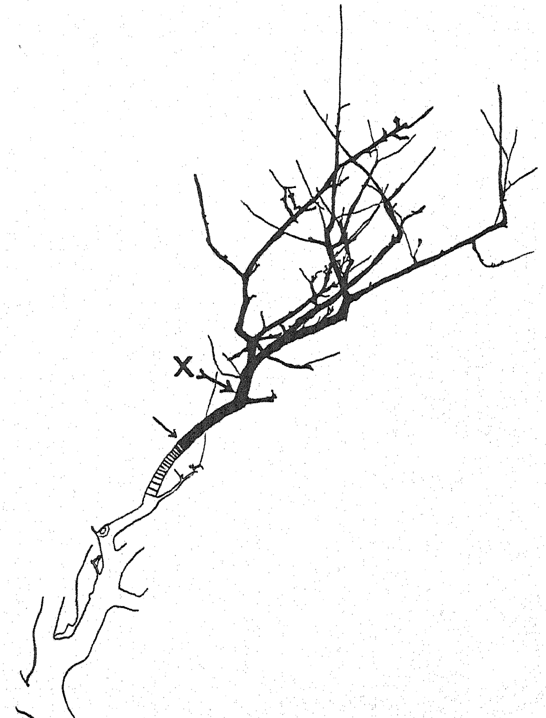


Fig. 34.

Injection of branches with few or no side branches near their base. X is suitable injection point.

safely be injected half-way between the desired lower limit of penetration and the branch tip. Text fig. 34 illustrates such a branch. The injection point is at X, heavy permeation is indicated by deep black and light permeation by shading.

The apparatus and the procedure will be described in detail because experience has shown that slight departures from the method here recommended may make an operation, which should be simple, rapid and satisfactory, appear difficult and even render it ineffective.

Apparatus and Procedure

A milk carton of wax-impregnated paper is, perhaps, the most convenient kind of container for the liquid (A. text Fig. 30) both because it is light and because its conical shape allows of many being " nested " together for convenience of transport. Two holes are punched near the top in the part of the circumference where the paper is double. String, thick enough to fill these holes, as nearly as possible, is threaded through them as shown in the figure. This kind of suspension will usually withstand all weather conditions except very high wind. A hole is made in the bottom of the carton with a long-handled reamer from the inside, which leaves a small external collar (F text fig. 30) protruding downwards from the base. The end of a suitable length of rubber tubing,* 2 mm. in external diameter, is moistened on the outside with rubber solution (care being taken to let none get inside the tubing), and inserted into the hole until it is flush with the bottom of the carton. Rubber solution is run over the outside of the base of the tube and of the carton collar, as well as a little on the base of the carton itself to form a sheath, and the carton is left upside down until the rubber solution is dry, when the rubber tubing is found to be fixed firmly into the carton.

Two pads, L and N, each measuring 4 cm. $\times$ 2·5 cm. for a large branch, or smaller for a smaller branch, are cut from rubber sheeting about 3 mm. thick. A small hole is punched in the centre of each and into each hole is inserted the end of a piece of glass tubing, $1\frac{1}{2}$ cm. long and 2 mm. in diameter, so that it protrudes about 1 mm. The small cap H is made by plugging one end of a short piece of rubber tubing with a piece of glass rod. A binding strip measuring about 10″ $\times$ 3″ is cut from a discarded inner tube of a bicycle tyre. For each injection in progress at a given time the above apparatus is necessary ; but all the items, with the possible exception of the binding strip, may be used many times.

In addition, each worker or group of workers requires a set of tools for use in drilling the holes through the branch. These consist of :

A piece of pure vulcanized rubber measuring about 4 cm. $\times$ $2\frac{1}{2}$ cm. $\times$ $1\frac{1}{4}$ cm.

A pair of calipers or a tape for measuring either the diameter or the girth of the branch.

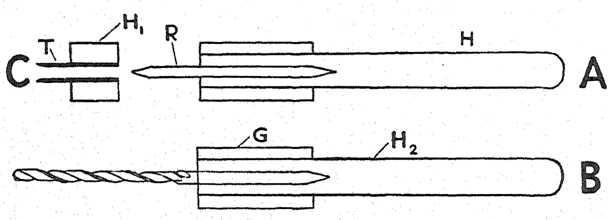


Fig. 35.

Apparatus for injecting small branches and trees.

For 1/16″ diameter holes : (for branches $\frac{1}{4}$″—$\frac{5}{8}$″).

A new metal twist drill 1/16″ diameter mounted as shown in text fig. 35 B in a wooden handle H_2 over which a piece of rubber tubing G is drawn to improve the grip.

A steel tube T (text fig. 35C) of such internal diameter that it will just slide freely over the drill. This tube is sharpened at one end from the inside and is provided with a handle H_1.

A tool A (text fig. 35 A) consisting of a rod R of 1/16″ diameter provided with a handle H at one end and sharpened at the other into a blunt chisel edge.

For $\frac{3}{32}$″ diameter holes : (for branches $\frac{5}{8}$″—$\frac{7}{8}$″ dia.).

For $\frac{1}{8}$″ diameter holes : (for branches $\frac{7}{8}$″—$1\frac{1}{4}$″ dia.).

For both $\frac{3}{32}$″ and $\frac{1}{8}$″ holes use is made of apparatus similar to that for $\frac{1}{16}$″ holes but larger.

For $\frac{3}{16}$″ diameter holes : (for branches $1\frac{1}{4}$″—$1\frac{3}{4}$″ dia.).

A $\frac{3}{16}$″ diameter Russell Jennings' wood boring bit. This is mounted as shown in text fig. 36D by cutting the blade from a ratchet screw driver and fixing the bit in its place.

A marking out tube (text fig. 36A).

A handled rod shown in two views in text figs. 36B and C ending in a blunt chisel edge with a central spike.

A rod of diameter just less than $\frac{3}{16}$″ provided with a handle at one end and at the other with a $\frac{1}{16}$″ twist drill soldered into it centrally and protruding from it about 1″ (see text fig. 36E).

For $\frac{1}{4}$″ diameter holes : (for branches more than $1\frac{3}{4}$″—3″ dia.).

Apparatus similar to that for $\frac{3}{16}$″ but larger.†

For branches of greater diameter than 3 inches the method to be described later for injecting whole trees is best used.

The following three points connected with the drilling of the hole are of importance :

1. In order to avoid unduly weakening the branch structurally and for quick healing, the hole should be as small as possible. As already stated, one of diameter one-eighth that of the branch is large enough to give complete permeation.

2. The cambium at each end of the hole should be damaged as little as possible so that healing may be rapid. It is better to remove a little extra bark and leave a neat wound than to leave a ragged one which will heal slowly.

* R.S.C. (red, steam-cured) valve tubing obtainable from Messrs. Dunlop Rubber Co., Cambridge Street, Manchester, at 12s. 8d. per lb. of approximately 90 yds.

† The above apparatus is made by the worker's past laboratory assistant, Mr. O. Usmar, by kind permission of the Director of the Institute for Research in Agricultural Engineering, Oxford.

3. The hole through the wood should be perfectly " clean ". A sharp new drill used without undue pressure results in the wood coming away freely in small pieces. The use of a blunt drill results in the detached pieces of wood being jammed together and rubbed against the wall of the hole, in this way closing up the cut ends of the conducting elements of the wood. Undue pressure in using a sharp drill has the same undesirable result.

Having decided on the approximate position of the hole, as already described, the actual position chosen should be the middle of the nearest piece of the stem above, in which there are no side-branches or other obstructions for a few inches.

The area within a distance of 1 cm. ($\frac{1}{2}''$) or more from each end of the hole should be smooth and free from excessive lichen ; if rough it should be made smooth with a " skarsten " scraper without causing unnecessary damage to the bark, and any moss or lichen should be removed with emery cloth or other cloth. The diameter of the branch at this point is measured with calipers and the set of apparatus of suitable size is selected.

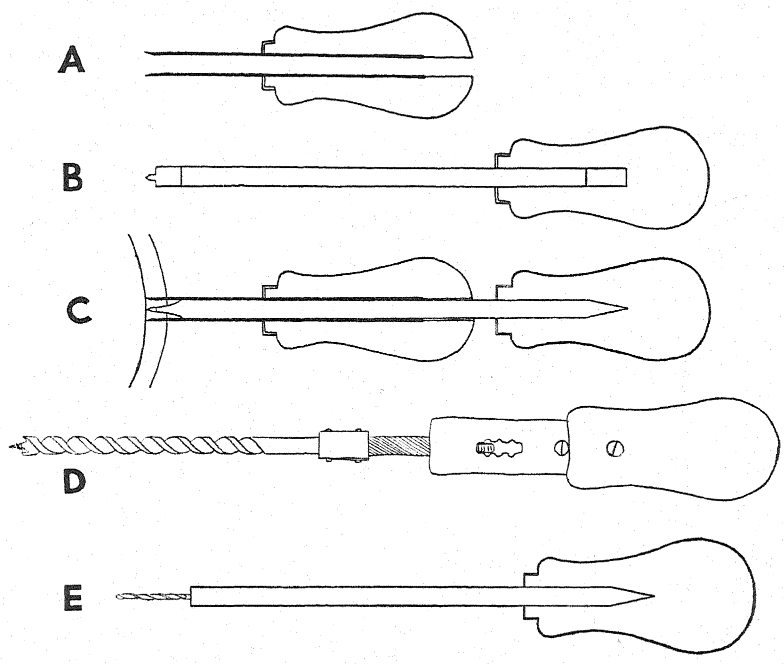


FIG. 36.
Apparatus for injecting large branches and trees.

For branches of diameter : $\frac{1}{4}''$—$\frac{5}{8}''$ ($\frac{1}{16}''$ *drill*).
,, ,, $\frac{5}{8}''$—$\frac{7}{8}''$ ($\frac{3}{32}''$ *drill*).
,, ,, $\frac{7}{8}''$—$1\frac{1}{4}''$ ($\frac{1}{8}''$ *drill*).

One end of the intended hole is marked out by pressing the tube T (text fig. 35C) through the bark just into the wood and holding it there while the chisel-ended rod (text fig. 35A) is pressed into the bark and rotated so as to detach it from the wood. The tube is removed and the bark is ejected by pressing the chisel-ended rod. The tube is replaced and the drill (text fig. 35B) is passed through it to start the hole. The tube is removed and the drilling of the hole continued. There is now a distance of $\frac{1}{16}''$ separating the drill from the bark ; the detached bits of wood pass through this annular space without damaging the bark ; further, the drill does not come in contact with the sticky material of the bark which would tend to cause small pieces of wood to adhere to it, these would become jammed against the sides of the injection hole and so would block the conducting channels. Further, this procedure prevents all risk of detaching the bark near the mouth of the drilled hole. A rubber block is held firmly against the point where the drill will issue from the branch ; and as soon as about $\frac{1}{8}''$ protudes the tube T is placed over it ; the drill is withdrawn while the tube is pressed so as to remove the ragged edge of the bark.

For branches of diameter : $1\frac{1}{4}''$—$1\frac{3}{4}''$ ($\frac{3}{16}''$ *bit*).
,, ,, $1\frac{3}{4}''$—$3''$ ($\frac{1}{4}''$ *bit*).

A depth about $\frac{3}{4}$th of the diameter of the branch is marked on the bit, the simplest way of doing this being to twist a wire pipe cleaner round it at the right point. One end of the intended hole is marked out by pressing the tube A (text fig. 36) through the bark just into the wood and holding it there while the tool shown in text fig. 36B is pressed into the bark and rotated so as to detach it from the wood. The tube is removed and the bark is ejected by pressing the rod. If the bark is thin the spike on the end of the rod will have made a shallow hole in the wood which marks the centre of the cylinder just cut from the bark. If the bark is thick the centre may be obtained by replacing the tube and pressing the spike into the wood while rotating the rod. The tip of the bit is placed in this hole, and the injection hole is now bored diametrically through the branch to the mark on the drill, i.e. so that it does not quite reach the bark on the other side of the branch. A smaller concentric hole is now drilled, in continuation of the first hole, through the remaining wood and bark with the rod which ends in a drill (text fig. 36E), undue damage to the bark being avoided by holding

the pad of rubber firmly against it. This small drill is removed, the tube A is slipped over the chisel-ended rod, the spike of which is inserted in the other end of the hole and the tube A is pressed through the bark to mark out this end of the hole, and while still in position the cylinder of bark is removed, as before, with the chisel-ended rod. The drilling of the hole to a uniform diameter is completed from this second end with the original first used drill.

Drilling the hole

The *modus operandi* described in detail above refers to all holes greater than $\frac{1}{16}''$ in diameter. Holes of $\frac{1}{16}''$ diameter may be drilled straight through the branch if the rubber block is held firmly against the bark on the opposite side, which need not become seriously damaged if the drilling is done carefully. It has been found advantageous to cut away any ragged edges of a wound in the bark with the smallest size of tube (see text fig. 35C), which will leave them quite smooth. Such a clean wound heals rapidly.

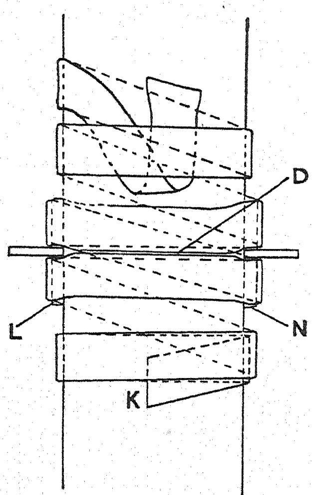


FIG. 37.

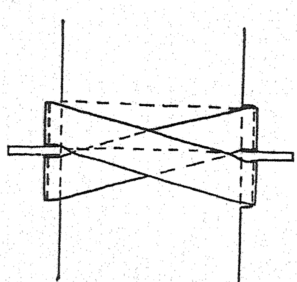


FIG. 37A.
Method for binding on tube-pads.

Binding tube-pads in position

One end (K, text fig. 37) of the rubber strip is placed $\frac{1}{2}''$ to $1''$ (according to the size of branch) below the hole D; the branch is held firmly in the left hand and the end of the strip is pressed firmly by the thumb of that hand against the branch. A few inches length of the strip is stretched with the right hand to two or three times its original length, wrapped round the stem and held under the forefinger of the left hand on the opposite side of the branch; the right hand grip is changed so that another length may be stretched and wrapped round the other side of the branch and over the end K to hold it firmly. The left hand may now be removed, the branch being steadied, if necessary, by placing the little and fourth fingers of the right hand against it; the remote, or left hand, tube pad L (text figs. 30 and 37) is placed so that the shorter protruding end of the tube is in the mouth of the hole in the branch; the stretched strip is wrapped over the pad on one side of the tube; and the second pad is placed in position in a similar manner. The binding is completed as shown in text fig. 37, the last turn being wrapped over the forefinger, with which a loop is pulled under the wrapping. This loop greatly facilitates the dismantling of the apparatus when the injection is finished.

The binding as described above, if carried out efficiently, nearly always makes a liquid-tight joint; but occasionally, for example, when the branch is of an inconvenient shape, a few more turns are necessary. It is sometimes an advantage to make the additional turns as shown in text fig. 37A. This method of binding concentrates the pressure on the parts of the pads most nearly between the two glass tubes, otherwise this position tends to be under less tension than elsewhere.

Pouring in liquid

With the tubing C (text fig. 30) hanging vertically, the liquid is poured quickly into the container. The amount of liquid lost before the end of the tube is pinched is negligible. The end is connected with the short tube in pad L; and as soon as the liquid flows out from the other tube (the one in pad N) the small cap H may be placed over it to stop the flow.

Should the liquid not flow from the second tube, in all probability one of the tube pads has been displaced in the binding operation and the end of the tube has become embedded in the bark and so closed; in such a case the binding of the tube pads must be unwound and the bind repeated with greater care, but if the instructions given above are followed carefully and if even tension is applied to the binding strip this difficulty should not be experienced.

When a series of injections is being carried out the whole operation should occupy on the average less than five minutes per branch.

Treatment of injection hole

Holes in apple trees made neatly in the early summer by the methods just described heal so completely during the same summer that they are hard to find again in the autumn even when left untreated; but electrical insulating tape or rubber adhesive plaster fixed over them would tend to keep out disease producing organisms. Such protection is desirable when there is reason to fear disease such as silver leaf, and then the holes may be disinfected effectively and harmlessly with a 1 per cent. alcoholic solution of 8-hydroxyquinoline.

Mechanical damage resulting from branch injection

Holes of the sizes suggested remove about one-twelfth of the cross sectional area of the branches at the injection point. With branches bearing a very heavy crop, this slight weakening may be enough to occasion a few breakages, but staking removes this risk. Generally speaking there is no risk of branches breaking or splitting as a result of injection. Sometimes, however, splits do start from the holes in the bark, especially if the trees are in a bad condition. No serious damage of this kind has so far resulted from injection and a split rarely starts from a properly drilled hole in a tree of average health and vigour.

Application of branch injections

Many trees in commercial plantations have ten, or even more, comparable branches which may be injected each as a separate unit. Each such unit may bear growths of various kinds, for example leader shoots, dards, fruiting spurs, and fruit. The method, therefore, is convenient for testing the general effects of a substance such as an artificial fertilizer, not only on the total amount of growth but also on how this growth is distributed. For example, one substance or mixture may tend to encourage "woody" leader growth, which rarely bears fruit, whilst another may encourage the types of growths which normally bear fruit. Further, the effects on the amount and quality of fruit produced may also be studied, including its storage quality and its resistance or susceptibility to disease attack.

In two preliminary notes, already published, the effects of injecting certain artificial fertilizers into the branches of spur-pruned pear trees (Srivastava and Roach 1937), and into those of apple trees which had not been spur-pruned (Sen 1937) were described. Sen also injected sugars and his work formed part of an investigation concerning biennial bearing. A. S. Horne of the Imperial College of Science, London, is at present collaborating with B. F. G. Levy and the writer in an investigation of the effect of the injection of artificial fertilizers and other substances on the susceptibility of the fruit to a rotting by a fungus *Cytosporina ludibunda*. H. Hill has used the method for testing in a preliminary way the effects of certain of the chemical elements that normally occur in fruit trees in minute amounts only. The results he has obtained with a nickel salt suggest that further trials with this element are desirable.

IX. Injection of individual branches together with their roots.

As already stated, in the injection method just described the liquid travels not only into the branch above the injection point but also to a less extent downwards, and if the hole is placed too low down there is a risk of liquid leaving the branch under examination and entering those contiguous of it. The lower the hole is placed on the branch, the greater is the amount of liquid which enters other parts of the tree. Not only does it invade other branches, but if close to the main crutch, and the soil be dry, the liquid enters the roots, sometimes penetrating even the finest rootlets at the ends of the longest roots (see also p. 56).

The planning of the type of injection to give the desired distribution depends, as in the types previously considered, on an accurate knowledge of the structure of the parts concerned, in this instance, the main crutch. Two simple experiments will make this point clearer.

If dye solution be introduced into a shallow hole bored into the wood of an unbranched stem of a tree it travels mainly upwards and downwards along the length of the stem, but it also travels a short distance circumferentially, and still less radially. The relative rates of travel in these three directions have been found experimentally to be of the order of 100, 10, 1, respectively. Further, if the injected stem be allowed to dry, the resulting cracks run in the same direction as the dye ; if the wood be split the splits also run in the same direction, and if thin strands are detached these too follow the same course. Since injected liquid follows the woody strands so closely their distribution in the main crutch must largely determine the type of distribution resulting from injections given in its vicinity.

The arrangement of the woody strands in the main crutch may be demonstrated by an experiment which is a development from the one last described. Instead of a single shallow hole a sufficient number are made at half inch intervals to encircle the whole main stem of the tree, and into these differently coloured solutions are injected for half an hour. This length

of time is enough if the day is fine and if the soil is dry. The tree is then dug up and its bark removed. As a result of the closeness of the holes to each other circumferential movement of liquid is less than when a single hole is bored, but longitudinal movement is not appreciably less. The whole stem of the tree becomes coloured in bands about half-an-inch in width which run from the roots along the main stem into the branches.

Text fig. 38 is a diagram of what may be seen when looking vertically downwards on the main crutch of a bush-trained tree treated in this way. The five branches were cut off just above the crutch and the cut ends of their stumps are seen at A, B, C, D, E. The main stem is drawn as if expanded at the base, where it has been cut off. The heavy lines 1, 2, etc. running from the cut ends of the branches to the base (i.e. the periphery of the figure) indicate the main directions of the woody strands. Strands 1 and 2 lie side by side in the main stem and also as they pass between branches A and E, but when they nearly reach the centre of the main crutch they bend, 1 going into A and 2 into E. The upper bent part of strand 2 lies side by side with that of strand 3, the lower end of which lies next to strand 4, which travels into branch D. As already stated, injected liquid moves most rapidly along the woody strands, but it also travels quite appreciably from strand to strand circumferentially. It is apparent, therefore, that liquid injected through a hole which cuts through strands 1 and 2 will enter, not only branches A and E, but also branch D. Strands such as these must, therefore, be avoided when the injected liquid is to be confined to a single branch. In other words, when the hole is made in the branch it must avoid the part nearest to the centre line of the tree if the injection of other branches is to be avoided; and if it is made in the main stem it must avoid the junction between two branches, or any point immediately below it (allowance being made for any twist in the stem). The risk of the injected liquid travelling into other branches than those desired may be reduced to a minimum by boring a single shallow hole as far away as possible from these strands, namely at E, on the outside of the branch; but such an injection through a single shallow hole does not always result in a complete permeation of the whole branch. When this is desired the hole is made as shown at FH, for it has already been shown that injection through such a hole usually results in a complete permeation of the whole branch. There is a slight risk of liquid travelling from the hole circumferentially to strands 2 and 3 and so out of the branch E into branches A and D. This risk is greatest in the inner annual rings because they are smaller and thus the actual linear distance to be travelled by the liquid from the hole to strands 2 and 3 is smaller; but this risk is lessened by the fact that the branches grow eccentrically, the rings being thicker on the outside than towards the centre of the tree, with the result that the hole, even if drilled diametrically through the branch, does not pass through the oldest rings at all. The hole, therefore, may be made with advantage slightly to the outside of the diameter and thus miss as many of the oldest rings as possible. All rings except the oldest one or two in twenty-year-old apple trees conduct injected liquid, but in the plum and other trees which have a core of dead non-conducting heart wood this precaution is not so necessary. Risk of leakage from one experimental branch to another is further lessened by the fact that each is being injected simultaneously and thus the tension in each is being reduced by approximately equal amounts. Untreated branches should, for this reason, be injected with water. Branches B, C, D, and E are alike in that the strands in continuation of them in the main stem are undivided, and all of them can be treated as just described, but the larger branch A possesses more than one set of strands in the main stem. In addition to strands 1 and 14 it has two small bands 6, 7, passing down into the main stem between branches C and D,

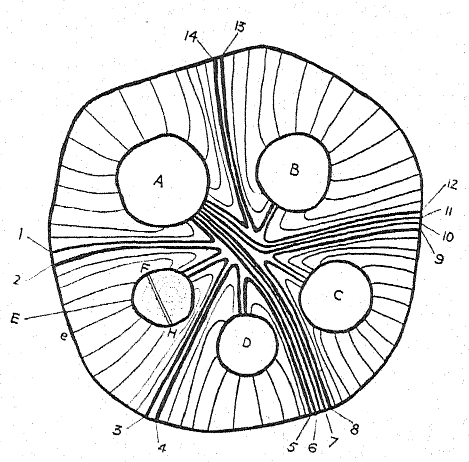


FIG. 38.
For description, see text.

and 2 smaller bands still, 10, 11, between branches B and C. If branch A be left uninjected bands of strands such as 6, 7 and 10, 11 will tend to prevent liquid passing from D to C or from C to B, and vice-versa. Such a branch is known as a " leader "; it is recognized by the fact that it is usually larger than the other branches, and commonly arises nearer the centre of the tree. Strands such as 6, 7 and 10, 11 also may usually be recognized at sight; thus branches such as A and B, D and E, E and A, which respectively have no such strands between them, meet each other at the crutch at a sharp angle; but branches B and C, C and D, either meet each other in a smooth curve or are separated by a flat area of bark obviously connected with the leader branch.

The tree just described as an example is the intermediate between those resulting from two types of training. The first is the well-trained bush tree common in the Eastern fruit growing districts of England in which the ideal is to obtain main branches of equal sizes, that is for all leader dominance to be counteracted so that neighbouring branches actually meet each other in the crutch and are not separated there by woody strands coming from a leader branch. In injection work it is particularly important in such trees that all untreated (control) branches which are to be used for comparison with those injected with nutrient or other substance, should be injected with water, to prevent them from withdrawing liquid from those specially injected. For the same reason the injection of all branches must be begun and stopped at about the same time, any of the reservoirs containing injection solutions that happen to become emptied before the end of the experiment being refilled immediately with water.

The second type is the well-trained, modified leader tree, common in Canada, in which neighbouring branches are always separated by strands from the leader branch. When these separating strands are well developed they constitute a sufficient barrier between the branches, and those " untreated " need not be injected with water. The leader branch will tend to absorb a little of each of the injected liquids, but, apart from this, it is of so much greater vigour than the other branches that it is not comparable with them for experimental purposes. Untrained or badly trained trees have to be taken each on its own merits, some being suitable for experiments of this kind and others quite unsuitable.

In modified leader and in untrained trees where strands from the leader branch separate all the injected branches from each other, the way in which the strands associated with these injected branches subdivide just above the crutch is of little or no importance from the present point of view, but may be of the utmost importance when bush-trained trees are being treated.

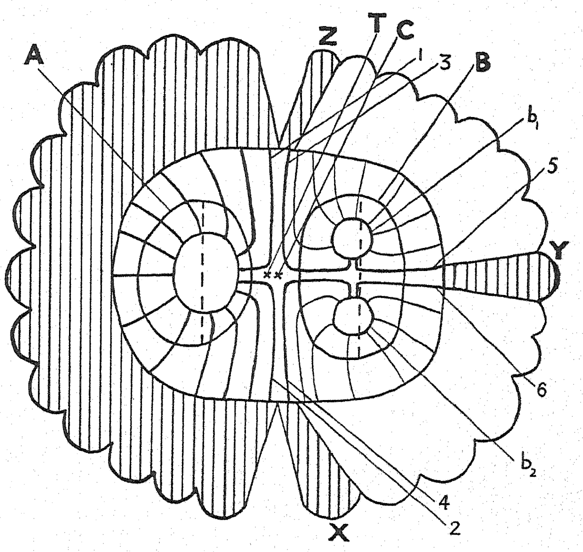


Fig. 39.
For description, see text.

Structures which make difficult the injection of individual branches together with their roots

Two examples will be given of branch structures the injection of which results in distribution of liquid in a way which may be most inconvenient from the experimental point of view. The first was discovered as the result of an early experiment carried out on a plum tree. This had two branches A and B (text fig. 39) nearly equal in size. They were injected through holes, indicated by dotted lines, A with 0·1% light green and B with 0·1% ponceau red 3R. Since light green travels about $1\frac{1}{2}$ times as rapidly and $1\frac{1}{2}$ times as far as ponceau red, it was expected that the parts Z, X of branch B nearest to A, would become dyed green; but in addition

to these parts a by no means negligible amount of foliage, Y, on the side of B opposite to A, also assumed the metallic green colour characteristic of the green dye. This result was at that time quite unexpected. Removal of the bark and partial dissection of the branches made the reason clear. Branch B, just above the crutch and the injection hole, divided into two sub-branches b_1 and b_2 which were in a plane at right angles to that of AB. It will be seen from the diagram how the free-moving light green dye solution passing down strands 1 and 2 on the side of branch A next to B could move into strands 3 and 4 respectively, since these lie side by side in the main stem. Having invaded strands 3 and 4, the dye ascended in them to the crutch between b_1 and b_2 and then ascended on the inner sides of both these branches. In a similar manner it crossed into strands 5 and 6 and descended them for some distance. The dyed foliage Y was carried by a small branch which happened to arise on strands 5 and 6.

Should this branch injection method be employed experimentally, occasional results of this kind are to be expected, and the question will arise as to whether the change on a particular branch or part of a branch on a remote part of a tree can have been influenced by a liquid travelling from a distant injection hole. Fortunately such a question can be decided without damaging the tree seriously. Using the same diagram for illustration, any liquid travelling from any part of branch A into any part of branch B must pass the centre C of the main crutch. A hole 1/32″ in diameter is, therefore, drilled at the point T through the bark so as to penetrate say $\frac{1}{8}$″ into the wood. Dye solution is dropped into this hole and into it is inserted a suitably tapered glass tube into which enough solution may be introduced to last for say one hour. This dye will mark out the region most likely to be affected by any injection carried out anywhere in branch A. The course of the dye can be followed by making as thin and neat a cut as possible in the bark of branch B, a foot or so above the hole T, along the suspected route until the dye is recognized; the process is then repeated. An alternative method is to allow the dye injection to proceed until the colour of the dye is recognized in the leaves. If dyes such as acid fuchsin, light green or patent blue are used little damage need be done to the branch.

A second and equally inconvenient type of distribution of strands is illustrated in text fig. 40. Such a condition of affairs has been observed in a number of trees. For simplicity the diagram is that of a tree with two main branches. One of these, A, subdivides just above the main crutch into branches a_1 and a_2. Branch a_2 is about one-third the size of a_1 and is so placed that one extreme edge of its strands touches the crutch centre, and the other passes through the injection hole. As the diagram suggests is possible, the injected liquid travels along strand 1, invades the crutch between a_1 and a_2, passes thence into strands 2 and 3, and from them into branch B. When the branches are arranged exactly as shown in the diagram, owing to the small circumferential distance involved, a considerable volume of liquid may pass from the hole in branch A into branch B; and this amount decreases the more the arrangement departs from that shown in the diagram.

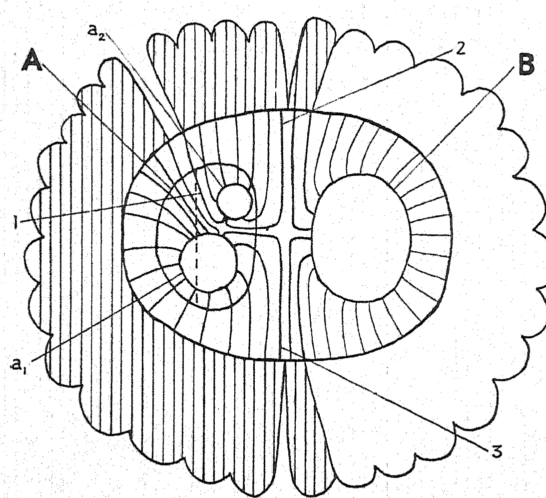


Fig. 40.
For description, see text.

THE INFLUENCE OF THE STRUCTURE OF THE ROOT CRUTCH ON THE POSSIBILITY OF INJECTING INDIVIDUAL BRANCHES WITH THEIR ROOTS

So far, attention has been concentrated on the main crutch, that is, on the region where the trunk divides to form the main branches; the structure of the corresponding region where

the trunk divides to form the main roots is also of importance. The same general considerations hold for this region as for the stem crutch. However, there is not a main root corresponding to each main branch and the roots seem to divide quite independently of the division into branches. In consequence the end of a hole placed in a satisfactory position in regard to the visible branch crutch may be in the worst possible position in regard to the usually invisible root crutch. It may for example, cut strands which pass down to the junction of two main roots and if this happens there is a risk of the liquid passing into the other main branches of the root, as may be seen in text fig. 41, in which the two strands O are actually cut by the hole (at a point higher up the trunk and not shown in the diagram) and 1, 2, 3, become permeated by circumferential movement of the liquid in the main stem, 1′, 2′, 3′ and 2′, 3′, by circumferential movement in roots P and Q, respectively. The main groups of strands between roots P and R, and R and Q respectively may both connect up with main branches other than the one intended, that is, the one "above" strands O. The actual volume of liquid leaving such a branch will be less than in a corresponding "leakage" across the branch crutch, because the resistance of the whole length of the trunk will tend to impede its flow. The risk of this leakage will be less in a "standard" tree with a long trunk than in a "bush" tree with a short trunk. Should leakage of this kind take place it will be greater the drier the soil, because, as will be shown later (p. 56), the movement of injected liquid into the roots is greater the drier the soil. Further, if one root is in drier soil than the others, more liquid will flow into it than into them. One consequence of this is that the amount of leakage from a given injection operation is dependent to a slight extent on the relative degrees of dryness of the soil surrounding the various roots, but this is a point of little more than academic interest. A test for a suspected leakage of this kind can be made by means of a small scale dye injection, the hole being made in healthy wood immediately below the main injection hole—allowance, of course, being made for any twist in the stem—and as close to it as possible. Strands such as the two labelled 1′ between P and R, and Q and R respectively are bound to be permeated, if any are, both by the experimental liquid and by the exploratory dye solution, but the two members of each of the two pairs of strands such as those labelled 3′, on the edges of the "leakage regions", may be respectively the one permeated by the experimental liquid and the other by the dye, if the relative distribution of soil moisture was very different on the two occasions. A somewhat larger injection hole may therefore be necessary to test for leakage across a root crutch than for one across a branch crutch.

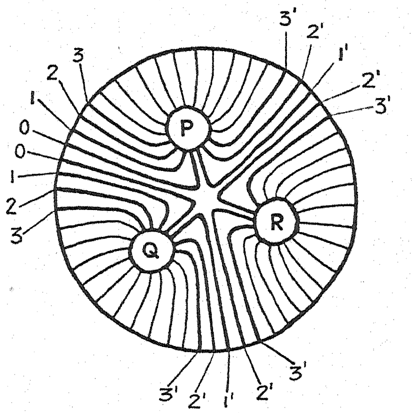


Fig. 41.
For description, see text.

Application of method for injecting individual branches together with their roots

As already stated, this method is particularly suited for experiments in which it is desired to inject some of the roots themselves as well as a branch ; otherwise it has no great advantage over the last one, in which the branches alone are permeated and not the roots ; and it has certain disadvantages. It has been described in detail not only because it has a definite although restricted field of use but also to make clear its disadvantages and how they may often be avoided.

The methods so far considered were designed almost entirely for diagnostic and analogous experimental work. The remaining method—that for injecting whole trees or plants—may also be used for diagnostic and experimental purposes, when it is desirable to compare units as large as whole plants with each other, but in special circumstances it may also be used for treating whole plantations on a commercial scale.

X. Injection of whole trees.

In the injection methods described in the earlier part of the paper the first and most essential object was to confine the introduced liquid within definite limits, and the same was true for the branch injections described later, except that the size of hole had to be suited to the size of the branch in order to ensure that every leaf above the hole should become permeated. When whole trees are to be injected, on the other hand, the primary consideration is to ensure that every branch shall have its full share of the liquid.

The methods worked out and now to be described vary according to the size and the shape of the tree. For trees trained as cordons the method described for branch injection (pp. 41-46) is quite satisfactory except that the hole should usually be bored a little below the level of the lowest branch. The same apparatus and procedure are suitable for bush trees with trunks up to 2 or 3 inches in diameter, except that the hole must be placed in definite relation to the branches. This point will be considered in the following section, after which certain modifications of the methods which are necessary for large trees will be described.

Position of hole

The correct location of the hole can be decided by referring once more to text fig. 38, already used in discussing the best position of holes through which to inject individual branches and their roots. For that purpose the strands indicated as thick heavy lines with numbers against them had to be avoided; since the inner strands of all the branches either meet or come close to each other in the centre of the crutch, if one of them is injected, there is a tendency for them all to become permeated. But what was undesirable for that particular purpose is highly desirable, indeed necessary, for the present one, although even these strands vary somewhat in usefulness for the object now in view. Liquid injected through a hole that cuts strands 1, 2 will pass directly into branches A and E; moreover, because the part of strand 2 in branch E lies side by side with strand 3, the lower part of which in the main stem lies side by side with strand 4, which in turn passes up into branch D, this branch also will become permeated. Liquid injected into strands 9 and 10 passes directly into branches C and A, across, into and up strand 11, then across this strand into strand 12, and so into branch B. Some hundreds of experiments have proved that injections through holes placed in such a position lead to practically uniform distribution of liquid throughout the branches, more uniform, in fact, than sometimes results from the application of manure to the soil and its natural absorption by the roots (Roach 1935a). The direction of the hole should divide the tree into as nearly equal halves as possible. From the work done the impression has been gathered that wide strands, such as 6 to 7, coming from a leader branch, tend to draw the liquid more strongly than from other branches. Although no definite proof of this can be given it is safer to avoid such strands as far as possible, and when the hole has to be made under a crutch entered by them, such as the one between B and C, it is best to inject to one side of them, namely, as already suggested, across 9 and 10, rather than parallel with them.

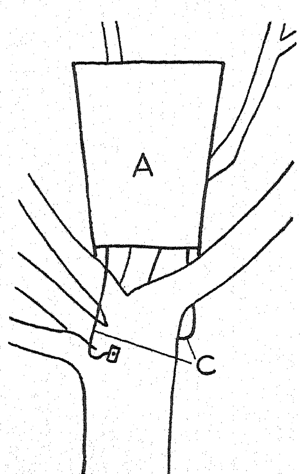


Fig. 42.

Injection of whole trees. The liquid is held in a reservoir A and led through rubber tubing C to the hole in the tree trunk.

Apparatus

Plywood buckets* A (text fig. 42) are used as reservoirs to hold the liquid. These are light and a number may be nested for storage and transport. Two small holes are drilled through the bottoms of the smaller size

* Obtainable from Messrs. Venesta Ltd., Vintry House, Queen Street Place, London, E.C.4, who describe them as type A and make them in various sizes. The ones of 1,219 cu.in. in capacity, costing 22s. 9d. per dozen, have been found most convenient for holding volumes of liquid up to 16 litres for injecting large trees. The size having a capacity of 619 cu.in., costing 18s. 3d. per dozen, are suitable for trees of medium size.

and four in the larger, if they are to be used for, say, full grown cherry or larger trees. The buckets are then made waterproof by immersion in hot paraffin wax until the stream of air bubbles practically ceases to rise. Into each of the drilled holes is inserted a short length of bicycle tyre-valve rubber tubing so that its end is flush with the inner surface, and this is made secure by inserting a short piece of glass tubing of such external diameter (0·25 cm.) as will press the tubing firmly against the wall of the hole, and thus hold the rubber tube tight. A $\frac{3}{4}''$ length of similar glass tubing inserted into the end of each of these short rubber tubes enables it to be connected to a longer one C (text fig. 43). Through the centre of the larger side of a rubber block D.* a hole, 0·6 cm. diam., is made and a smaller one, 0·4 cm. diam., is drilled at right angles into it from the end of the block. A short length of glass tubing E is inserted in the end of the rubber tubing C, and this is inserted into the smaller hole in the block, rubber solution being used both as lubricant and to seal the joint. The glass tubing helps in this respect, and also prevents the walls of the hole from collapsing under pressure. The block makes a water-tight joint with the bark F (smoothed beforehand if necessary) on one face, and, on the other, with a rubber pad H (cut from an old motor tyre inner tube) which makes a water-tight joint, with the shank of a No. 8 wood screw† J, that passes through a hole punched in it the point of the screw is eventually driven into the blind end K of the previously drilled hole, and supplies the grip which enables its head to hold a block of metal L so that this, in turn, holds the two rubber pieces D, H, together and presses the block D sufficiently tightly against the bark to prevent leakage. The liquid flows through the rubber

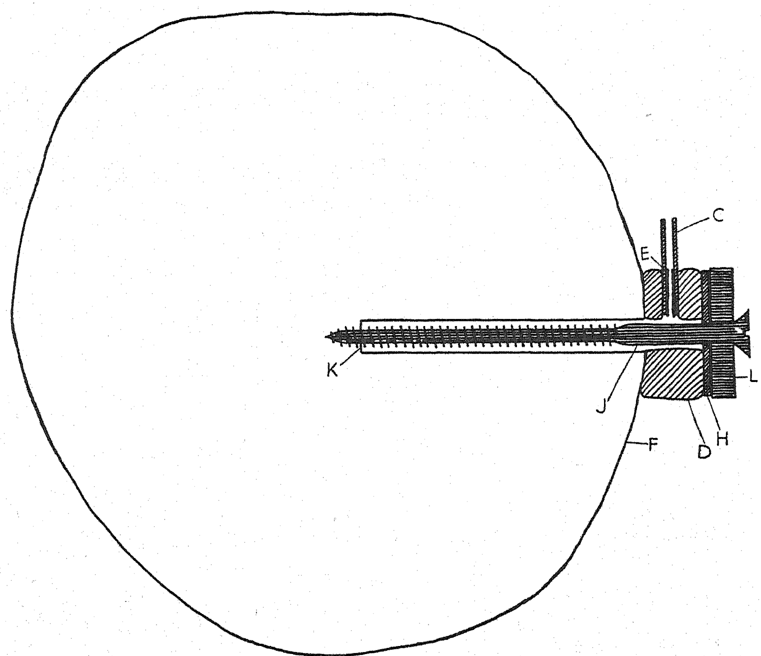


FIG. 43.‡

Injection of whole trees. The liquid enters through the rubber tubing C and passes into the space between the screw and the wall of the hole in the tree trunk. For further description, see text.

* Rowney's "R" grey rubber, measuring $1\frac{3}{4}'' \times 1\frac{1}{2}'' \times \frac{1}{2}'$, obtainable from most stationers at 3d. per piece. These last three seasons or more.

† The wood screws after being allowed to rust slightly are plunged into hot paraffin wax to give them a film of wax to render them resistant to attack by the injected liquids.

‡ The above apparatus is satisfactory but some simplification and increased rapidity in use are desirable. In early experiments a rubber stopper or a tapered steel or glass tube was inserted into the hole. If inserted into the bark there was a tendency for the bark to split and if into the wood the vessels in contact with the stopper or tube were closed; and because of the slow radial movement an outer ring of wood nearly as thick as the plugged vessels was rendered useless for conducting injected liquid. This is of little consequence in, for example, an apple tree in which practically the whole thickness of the stem conducts injected liquid. But in the plum only a comparatively thin ring of sapwood conducts, and consequently any such closing of cells is to be avoided. Attempts are being made to improve the apparatus.

tube C, between the shank of the screw (see below) and the rubber block D and then into the space between the screw and the wall of the hole in the tree trunk.

The holes are drilled with apparatus of the types illustrated in text figs. **36** ABCD but of suitably increased size.

In addition, a supply of glass rods (about **1·5—2·0** in. long, and **3** mm. diameter) with rounded ends, is required to plug the ends of the rubber tubes temporarily.

Procedure

When many trees are to be injected it is advisable to decide on the positions for the injection holes beforehand, and convenient to mark them with metal-handed push-pins (available from Messrs. Kodak). The heads, being made of white metal, are conspicuous against the bark. The exit tubes from the reservoirs are placed in the crutch, or suspended from a branch so that the tubes will be in convenient positions in relation to the holes, and the liquid is then poured in. After all the reservoirs have been placed in position and filled, the actual injection is carried out. It is an advantage to keep all the tools in one shallow box, and sufficient "parts" such as rubber blocks with tubes attached, screws of suitable sizes, etc., in another. These boxes should be divided into convenient compartments so that each tool and set of parts has its proper place, and they should have handles for carrying. The girth or diameter of the tree is first measured and the table below consulted:

TABLE 2

LENGTHS OF HOLES AND SCREWS FOR TREES OF VARIOUS DIAMETERS AND GIRTHS.

All measurements are in inches.

Diameter.	Girth.	Depth of holes.	Length of screws.
3	9	1	2
4	13	1½	2½
5	16	2	3
6	19	2½	3½
7	22	3	4
8	25	3½	4½

The depth of the hole required is marked on the boring bit by a wire pipe cleaner twisted round it. A screw of the proper length is pushed through a metal piece L, a rubber pad H and a rubber block D. The hole is now bored. The glass plug is removed from the nearer short tube protruding from the bottom of the bucket and the rubber tubing is connected to it by means of the short length of glass tubing. The end of the screw is placed temporarily in the hole in the trunk so that the rubber block may be adjusted quickly in position with its hole fitting accurately over that in the trunk. The screw, with the attached metal piece L and rubber collar piece H, is now removed and held in the right hand. The thumb of the left hand is placed firmly over the bottom half of the rubber block and over the hole in it, while the top half is pulled away slightly from the bark to allow the liquid to fill the hole and displace all the air. The screw, with its metal piece and rubber collar piece, is pushed into the hole and driven with a ratchet screwdriver until liquid ceases to ooze. The amount of liquid lost during the operation is negligible. If all external moisture be now removed with a cloth a leak, if it exists, may be detected in a few seconds and remedied, usually by tightening the screw. The time taken after the liquid has been poured into the reservoir, to complete the actual operation, is five minutes a hole, i.e. a total of ten minutes for most large orchard trees.

Degree of importance of position of hole

The principles underlying the proper placing of the injection holes have already been described and the desirability of placing them as nearly as possible in the ideal positions has been emphasized. A comparison of the results of one experiment carried out before these principles were worked out with those of one carried out after they had been elucidated will be instructive and will now be made.

An early experiment in which the hole was bored in a wrong position

A thirteen-year-old apple tree was bored through its main stem just below the crutch with a ¼″ bit, and the hole was connected with rubber tubing to a reservoir containing 10 litres of 1·01% solution of potassium nitrate. The whole of this was absorbed during the hot dry afternoon of **17-6-32**. Scorching of much of the foliage was apparent late the same evening, but was much more evident on the following day, when the affected areas of the leaves had changed from " dried

up " green to brown. This scorching will be dealt with in greater detail in another connexion later on. It was most severe in the leaves of two branches directly above the two ends of the injection hole, and least so in two branches most remote from the hole.

Two months later, new leaves that had developed by that time were large, thick and of a strikingly healthy dark green colour all over the tree, in marked contrast to the small thin old leaves of unhealthy colour on the tree prior to the injection. The whole tree from being the most unhealthy-looking specimen in the whole plantation had been changed, as a result of the injection, into the healthiest looking. In the autumn the extension growth both for 1931 and 1932 was estimated on nineteen shoots which had remained unbranched in both these years. The results were as follows :—

Total extension growth made by 19 shoots in 1931 and 1932 in cm.			ratio 1932-31.
8 shoots on two branches over hole	161	287	1·8
5 ,, one branch nearly over hole ..	120	207	1·7
7 ,, two branches remote from hole ..	172	256	1·4

Thus the two branches, the foliage of which was most severely scorched immediately after injection eventually increased most in length, whereas those, the foliage of which was least damaged, eventually increased least in length. Hence the same lack of uniformity of distribution of the solution along the branch was indicated both by the immediate damage produced and later by the increase in growth. This is an example of the most unsatisfactory distribution ever likely to result from an injection through a hole made in the wrong position in a tree with five main branches arising from the main stem at about the same height. The lack of uniformity in the distribution resulted almost entirely from the hole being directly beneath two branches instead of lying between them and rather low down.

A later experiment in which the holes were bored in correct positions

As an example of the type of results obtained when the holes are properly placed one may be mentioned which has already been the subject of a progress report, (Roach 1935a). In this experiment eight, twenty-one-year-old, bush apple trees in a commercial plantation were injected with a solution containing equal weights of dipotassium hydrogen phosphate and urea at rates which varied between 10 and 50 lb. per acre for each substance. After the injection one branch of each of three trees showed an easily visible, but not serious amount of leaf damage, and two others were slightly damaged ; in the remaining five trees damage was negligible. In each of the first three trees the end of the hole was not accurately on the dividing strands between two branches, but was on the damaged side of the branch. The main stem of each of these three trees, which was about 10 cm. in diameter, was bored with a single hole which passed diametrically through it, and the slightly faulty results were due to the difficulty of " aiming " at an invisible point on the farther side of the stem. This difficulty does not arise in the newer method described on p. 52.

In the autumn differences in growth were apparent between these eight trees, eight other comparable untreated trees, and a further eight trees in the same plantation which were injected with water only. These varied from a barely detectable difference in growth in the tree receiving the smallest amount, to a fourfold increase of growth in the one that was injected at the rate of 27 lb. per acre. As far as could be judged from a careful visual examination of the injected trees all the branches on each were equally invigorated.

The imperfect distribution of the potassium nitrate in the first experiment, as judged by the damage and the subsequent growth response, is typical of the irregular distribution of dye and of various salts (as judged by the leaf damage (see p. 62)) in many scores of the earlier experiments carried out before the importance of the position of the hole was realized. The results of the experiment just described are typical of those obtained in some hundreds of the later injections. The position of the injection holes must therefore be selected with care if the best results are to be obtained.

Exclusion of air from injection hole

In this section will be considered a precaution which has been exercised by a number of past workers but the importance of which is still not established.

In transpiration experiments, etc., it is usually recommended to cut off the plant organ under water to prevent the entrance of air into the wood vessels. Shevyrev (1894) seems to have been the first to advocate the exclusion of air from cut surfaces that are to be injected ; but it has been shown (see below) that his reasons are not conclusive, and he does not appear to have submitted them to definite experimental tests. This is true also of a number of other workers, e.g. Mokrzecki (1903) Rumbold (1915, 1920) and Storey and Leach (1933), who have followed his lead in this respect. Roth, on the other hand, as already pointed out, obtained remarkably rapid injection through holes that had been open to the air for as long as thirty-six hours before liquid was introduced. Other and later workers have also found the exclusion of air unnecessary, for example, Collison, Harlan and Sweeney (1932).

The attempt to exclude air from the injection hole complicates the actual operation so greatly that for its justification the rate of injection or the thoroughness of permeation would have to be at least doubled. In a number of the earlier experiments leading up to the work described in this paper, and in some done later, comparative tests were carried out sufficiently carefully to reveal differences of this magnitude if they existed, but in none was a definite advantage secured by excluding air. Hence, it was decided not to introduce this complication until there was a necessity for doing so.

An observation frequently made in the course of these experiments has a limited bearing on this subject. Until the present season (1937) the liquid was often siphoned from the reservoir into the injection hole. Occasionally, when a number of reservoirs were found empty and were refilled, it was observed that the siphons often "started themselves". Under the exceptionally dry conditions of soil and atmosphere in late July 1931 a supply tube was disconnected to make sure that it contained no liquid and was again sealed to the injection hole. The free end was then placed in the liquid in the refilled reservoir situated a foot or so above the hole. In about a minute the liquid travelled along the tube to the hole. Air must have been drawn into the hole (under slightly less than atmospheric pressure) to raise the liquid in the tube over the edge of the reservoir and thus start syphonic action. This phenomenon was observed twelve times in a number of trees in a single day. Evidently trees can sometimes take in air through injection holes, and they have been proved to do this for many hours on end, but while doing so they also absorb liquid in large volumes through the same hole. This phenomenon has been observed only when both the soil and the atmosphere were comparatively dry. The explanation given by Sen and Blackman (1933) and by Dickson and Blackman (1938), of a similar phenomenon probably also applies to this one.

Occasional failure to become injected

As already stated, faulty drilling of holes, especially when temporary clogging results from the use of a blunt bit, seems to have been the commonest cause of failure in the past. Even when holes and cuts are made cleanly failures to bring about injection are still sometimes encountered.

No difficulty has yet occurred in injecting adequate quantities of liquid by any of the methods described except that used for whole trees. No difficulty was experienced even with this method until 1936 ; but for periods of some weeks during the summers of 1936 and 1937 injection was sufficiently unsatisfactory to warrant some attention to the matter. A few experiments will therefore be described which suggest certain tentative conclusions as to the conditions under which unsatisfactory injection may be expected and which show how it may be avoided.

The interveinal method works satisfactorily even when applied to trees growing in a culture solution in a greenhouse. Injection of branches by the method described on p. 44 is slower when the soil and the atmosphere are both nearly saturated with moisture than when both are relatively dry, but on no occasion as yet has injection been inconveniently slow. When, however, whole trees have been under treatment, the soil and atmosphere both being moist, injection has commonly been inconveniently slow, whilst on a number of such occasions there has been no injection. The tendency to slowness of injection seems to increase as the distance of the

injection point from the foliage increases, and as its distance from the root decreases. This is in harmony with the increase in negative tension in the sap from the root to the leaf.

Influence of soil moisture on injection

When the soil is comparatively dry the root-sap of an apple tree in full foliage is in a state of tension, as is proved by the fact that dyes injected in solution under negligible pressure commonly reach the finest root tips. The impression was gathered from a number of experiments that the proportion of dye in the roots to that in the upper part of the tree was smaller with the soil wet than with it dry

An experiment carried out during the summer of 1932 is of interest in this connexion. A fortnight prior to its start, as a result of drought, the soil and sub-soil were comparatively dry down to the underlying rock, subsequently heavy rain fell and the uppermost foot of soil became moist. A large apple tree in full leaf was injected with dye solution for about three days through a hole in its trunk by an early method. The tree was then beheaded and the roots excavated. A few of the smaller roots which went nearly straight down into the dry soil had become deeply dyed to their tips, in striking contrast to the apparently similar roots that were entirely in the moist top layer of soil which were not dyed at all. The appearance of the main roots was even more striking. A cross section of each of these near the trunk was partly dyed and partly uncoloured.

These and their sub-branches were followed downwards until the cross-section of each was either completely coloured or entirely uncoloured. The completely coloured roots always ramified in the dry soil and the uncoloured roots in the wet. It is apparent, therefore, that although this injection was allowed to proceed for three days, only the rootlets in dry soil became permeated.

A few further points of interest may be mentioned here in passing. The movement of dye to the very tips of the roots suggests that some of the water in which it was dissolved must have left the root tip and entered the soil, since water usually moves more freely than the dye dissolved in it ; this further suggests that the normal movement of water, and possibly of simple salts dissolved in it, from the soil into the roots, may be a reversible process. This movement of substances to the root tips, and possibly through them, is of some theoretical interest as it suggests experimental and practical possibilities ; for example, the thorough injection of roots when the soil is dry suggests that they could be killed by this means—if desired—more efficiently under these conditions than at other times. This increased efficiency might make possible the substitution of higher concentrations of substances such as artificial fertilizers, for the highly poisonous substances commonly used for killing trees.

The fact that the movement of injected liquid is influenced by the moisture condition of the soil, coupled with the known effects of climatic conditions on the rate of transpiration, explains to some extent the difficulties in ensuring adequate liquid injection when both the soil and the atmosphere are moist.

Injection under pressure

Injection of liquid under pressure obviously suggests itself as a possible method of overcoming the difficulties referred to above. Before dealing with the direct tests made in this connexion a few early experiments will be described. These were carried out in the summer of 1932 on trees in full leaf when both soil and atmosphere were dry, and their object was to compare under these conditions the rate and extent of injection under (i) small negative heads of water, (ii) heads of water up to 2·5 m., (iii) pressures of many atmospheres.

(i) *Small negative pressures*

A few experiments were carried out to determine how far trees could be injected with liquid from a vessel placed below the injection hole—for example, standing on the ground. It was found that, as long as air was not allowed to leak into the hole and break the water column, such injection proceeded practically as rapidly as that under small positive heads of liquid ; but a small positive head was found a distinct advantage because it is easier to prevent liquid

escaping from a system under slight positive pressure than to prevent air entering one under slight tension.

(ii) *Pressures up to 2·5 metres*

For this experiment two similar and equal-sized branches, arising on opposite sides of the main stem of an apple tree, were pierced with ½ inch holes at a distance of 50 cm. above the main crutch. Each hole was connected with rubber tubing to a reservoir containing dilute dye solution. The level of the liquid in one reservoir was about ½ metre, in the other about 2½ metres above the hole. In two days each branch had become injected with about 5 litres of 0·05% light green dye solution. The experiment was then repeated with 0·05% ponceau red solution on two similar branches of the same tree which lay mid-way between the first two branches. It was found in both cases that the height of the liquid in the reservoir, i.e. the pressure, had no appreciable effect on the rate of injection, the high pressure branches taking ½ litre more of the light green solution but ½ litre less of the ponceau red solution than the low pressure branches. The foliage on all the injected branches became dyed sufficiently to be easily distinguishable from a distance. The foliage on other non-injected branches did not become visibly affected, but dye could be detected in the stalks of most of their leaves when cut.

Cross sections of the branches above and below each of the injection holes each showed a heavily dyed bar about the width of the hole with a less deeply stained region on each side. In the two low-pressure injected branches there was a region on one side of the dyed area quite free from dye for a distance of some 20 cm. both upward and downward from the hole. The heavily dyed bar tended to broaden and become less distinct as the distance from the hole increased, and this occurred more rapidly in the two branches injected under high pressure than in the other two. These were the only differences noticed between the branches injected under high pressure and those injected under low pressure. All woody tissue more than 50 cm. above the holes became completely permeated whether the pressure was high or low. The four bands of dye were to be traced down the main stem without coalescence and could be seen in the roots for about a metre from the base of the trunk. Further experiments in which pressure heads of 2·5 metres of water were used will be mentioned later.

(iii) *Pressures of a few atmospheres*

With the permission of the workers concerned a description will be given of a method in use at the Sub-tropical Horticultural Research Station, Nelspruit, Eastern Transvaal.

"The apparatus consists of a Primus blowlamp from which the whole of the flame control portion except about 2 or 3 inches of the supply tube is removed. To this stub a length of rubber pressure tubing is attached. Into the other end of the rubber tubing a short piece of glass tubing is inserted, which passes through a rubber stopper of the correct size.

"A hole, ⅜ inch for small trees and branches to ⅝ inch for thick branches, is drilled into the tree or branch to be injected, the stopper inserted firmly into the hole and the glass tubing (which is attached to the rubber tubing leading from the blowlamp) filled with the solution, inserted into the hole in the stopper after the tube has been filled with the solution. Pressure is now applied by working the pump of the blowlamp.

"We have been using a maximum of 500 cc. of solution (the capacity of the container) per injection, with a maximum pressure of 40 lb. per square inch. Concentrations vary from ·1% to 5%, depending on the toxicity.

. . . .

"It takes on the average one hour to inject the 500 cc. One person can just handle 8 of the 'injection units' and can do about 60 injections a day."

(iv) *Pressures of many atmospheres*

In these experiments a pump of standard pattern* fitted with a nozzle tapering from 1·1 cm. to 0·6 cm. in 3 cm. was used. The plunger was 1 cm. in diameter and a pressure on it

* A Ki-Gass Petrol Mist Injector made by Messrs. Rotherham & Sons, Ltd., Coventry.

of less than 2 lb. exerted a pressure of 1 atmosphere on the liquid ; a pressure of many atmospheres, therefore, was readily obtainable. With a brace and a $\frac{1}{4}''$ bit a hole was made 30 cm. above the ground not quite through the 11 cm. diameter stem of a thirteen-year-old apple tree. The nozzle of the pump was pushed firmly into the mouth of the hole after the air had been displaced by N/100 sodium thiosulphate solution and pumping was started ; 800 cc. of solution was injected in about 3 minutes. Distant twigs cut immediately after the injection clearly oozed liquid, but this ceased in about half an hour.

One injection was given on 19/5/32, and a further one on 15/6/32. An attempt to use the same hole failed as the resistance was too high. A few forcible strokes of the pump, made in an effort to clear the ends of the conducting vessels were followed by a split 15 cm. in length in the bark on the opposite side of the trunk, the bark being loosened from the wood for about 1 cm. on each side of the split. A fresh hole was then made just below the crutch, i.e. 27 cm. above the first hole, and at right angles to it. On pumping gently there was no oozing either from the old hole or the split, but on pumping harder this occurred first at the hole and then at the split. A volume of 4,000 cc. liquid was injected late in the afternoon. Oozing took place alone from cut twigs along the length of all the main branches for about one-quarter of their length, but there was no oozing from more distant twigs. No damage to the foliage resulted from this injection, hence the degree of uniformity of distribution could not be judged easily.

By kind permission of Mr. J. Chambers, of Southfleet, the same method was used on 29/5/32 to inject five twenty-year-old apple trees with water, 5% potassium sulphate, $2\frac{1}{2}$% potassium sulphate, 5% potassium monohydrogen phosphate and $2\frac{1}{2}$% potassium monohydrogen phosphate, 3 litres of one of these solutions being injected into each tree. On 20/6/32 the two trees injected with 5% solutions showed serious foliage scorching and the terminal wood of some of the branches had been killed ; the trees injected with $2\frac{1}{2}$% solutions showed slight leaf scorch but no wood damage. In each of these trees the damage was most severe in branches arising vertically above the hole. The distribution, therefore, was not quite uniform. This uneven distribution in all probability resulted from the holes not being properly placed with respect to the branches (see p. 52) ; consequently, even for injection under high pressure, the position of the hole is of great importance.

The results of a number of similar experiments carried out on apple and plum trees both in summer and in winter pointed to the same conclusion, namely, that injection pressures of less than an atmosphere had a negligible effect on the rate of injection and that, to cause any marked increase in that rate, a pressure of the order of 10 atmospheres is necessary ; with such pressures there is some risk of causing splits in the main stem and of detaching the bark from the wood near such splits.

These experiments have been described at this stage because their results are in harmony with the conception indicated earlier by which the movement of liquid in the tree, under the influence of large forces, is impeded by correspondingly large frictional resistance. Obviously such movement would be influenced by an external pressure only if it were large. But the conditions of the above experiments are not the same as those which make for unsatisfactory injection. The high frictional resistance is substantially the same for both but, whereas dry soil and atmosphere tend to place the liquid system of the trees in the above experiments under great tension, yet, when injection was unsatisfactory, moist soil and atmosphere must have brought about much lower tensions. The effect of small liquid pressure heads therefore appeared worth trying.

(v) *Further experiments in which pressures of 2·5 metres were used*

In a 1936 experiment (Levy and Roach 1937) on apple trees, injection did not proceed at all with some of them while with others it was slow. On some of the trees which did not become injected at all, the reservoir was raised until the liquid was being injected under a head of about 2 m. (6 ft.) ; but none of the trees so treated became injected. Dr. F. G. Anderssen, in a letter dated 2/3/37, from which he has kindly allowed quotation, states that " the ordinary methods of injection are not effective " for injecting into the trunks of citrus trees " as the solution is not absorbed by the tree ; when, however the solution is placed under a pressure

of about 5 lb. per square inch it is absorbed readily ". Apparently, therefore, this large liquid tension and large frictional resistance may happen to be balanced so that a small external pressure is sufficient to force in liquid which otherwise would not become injected. It will be noticed that this pressure, which corresponds to a head of 10 ft. of water is somewhat larger than that tried by the writer and his co-worker.

In a number of experiments on apple trees, including those just mentioned, fresh holes were bored without causing any appreciable improvement in injection.

Similar difficulties were encountered during the early part of the summer of 1937. Although a number of trees became injected satisfactorily, others in the same plantation became injected only slightly or not at all when treated as described on pp. 52-54. Levy, however, discovered that after these trees had been injected with a small volume of liquid under pressure by the method described on p. 58 (iv), injection occurred in the usual manner as soon as liquid was led into the holes again. He obtained similar results on eight apple trees in which injection had either ceased or had not occurred at all. These facts will be published in greater detail in due course, but permission has been given to mention them here.

Applications of whole tree injection

The injection of whole trees is being used experimentally for purposes similar to those for which branch injections are used, and it is to be preferred to the branch method when a large experimental unit is advantageous. As an example the preliminary work already reported (Hulme, Levy, Roach 1937) may be given : the object was to vary the composition of a number of lots of fruit, each consisting of several bushels, to allow of chemical, physiological and storage tests being carried out on them after picking.

In some plantations individual trees may be selected which resemble each other more closely than do the separate branches of any single tree. In these circumstances the whole tree method is obviously preferable to the single branch one.

Possible commercial applications of this and similar methods will be considered later (pp. 68-71).

DETERMINATION OF DISTRIBUTION OF INJECTED LIQUIDS

The trustworthiness of all the methods described in this paper depends largely on that of those used to trace the distribution of liquid resulting from experimental injections; and this will be true also of any attempts which may be made in the future to modify them for other purposes or to adapt them to other plants. The real value of any injection method, at least from the experimental point of view, may be judged from the extent to which the resulting distribution can be predicted and controlled; and the final test is to ascertain how far prediction coincides with the result of the injection as judged by the distribution of the effects produced by the substance injected, for example, improved foliage colour, increased growth, freedom from disease, etc.; but in the earlier stages, much more rapid methods such as the use of dyes have obvious advantages.

The employment of dyes was the earliest of these rapid methods used. Next a substance such as a lithium salt was used which is easily detected spectroscopically, or a cyanide, which is readily recognized by chemical means. Lastly the substances suitable for main experiments, such as were employed in some of the writer's earlier experiments, may be utilized in such concentration and quantity as to produce slight leaf damage which betrays the distribution of the liquids.

Use of dyes

Dyes have the obvious advantage of easy and almost immediate visibility and give more rapid results than any of the above methods. As shown already some idea of the type of distribution resulting from a leaf injection with a dye may be obtained in a quarter of an hour, and the distribution can be mapped in an hour. When main branches or whole trees are injected with dyes, the distribution may be seen readily after a few hours if injection is rapid. These remarks, however, apply only to certain dyes.

Reference has already been made to the work of Goppelsroeder (1889, 1901). He found that those dyes which travelled in solution most rapidly up blotting paper strips dipped into them also ascended most readily leafy shoots whose cut ends were placed in solution of the dyes. Another method used by him for comparing dyes consisted in dipping a paper strip into a mixture of two dyes. For most mixtures the two colours were combined at the bottom of the paper, but above it the colour suddenly changed to that of the dye which travelled the faster. This method of mixing dyes in pairs was used by the writer to compare a number of dyes both with strips of blotting paper and also on plant material For the latter two methods were used: in one the cut ends of leafy shoots were immersed in the mixtures while in the other the mixtures were dropped into shallow holes bored in a tree trunk. The pure dye solutions were also dropped into similar shallow holes. All these methods gave substantially the same results which were in harmony with those obtained with the same dyes when used for injection work. In the following table 23 dyes are placed in the order determined by these tests, beginning with those which travelled most freely and ending with those which hardly travelled at all.

TABLE 3

1.	Acid Fuchsin.	9.	Azo Geranine.	17.	Methyl Orange.
2.	Light Green.	10.	Lissamine Fast Red.	18.	Methyl Violet.
3.	Lissamine Fast Yellow.	11.	Coomassie Yellow.	19.	Neutral Red.
4.	Patent Blue.	12.	Solway Blue.	20.	Congo Red.
5.	Ponceau Red.	13.	Alkali Blue.	21.	Crystal Violet.
6.	Water Blue.	14.	Erythrosin A Eosin.	22.	Fuchsin.
7.	Tartrazine.	15.	Erythrosin B Eosin.	23.	Bismark Brown.
8.	Amaranth.	16.	Methylene Blue.		

Case (1938) has found Orange G a satisfactory red dye. Methylene blue, which was found practically useless by the present writer for apples and other trees, has been used

successfully by other workers for other plants. The dyes found most suitable in the present work were acid fuchsin (red), light green and patent blue. The last two were first used for this purpose by Harvey (1930), and the second of these he listed under its synonym "brilliant blue". All three dyes are nearly harmless in 0·1% solution to living plant cells, the least harmful being patent blue, which is probably the most generally useful for injection work. Only highly purified dyes should be used, otherwise there is a risk of the wood vessels becoming choked by the impurities, some of which seem to become precipitated slowly from solution on standing. The first two dyes, as supplied for microscopical work, and the purest grade of patent blue, as supplied by chemical firms, all give satisfactory results.

A number of distributions first determined with these three dyes have since been verified by the results obtained with nutrient solutions. These include results from the interveinal method on the apple (see p. 24), and the broad bean (by H. Hill who will publish full details shortly); from the leaf stalk method on the apple (Roach and Levy 1937); from the shoot tip method on the apple (this will be mentioned again shortly) and cherry (Roach and Levy 1937); and from the individual branch method on the pear (Srivastava and Roach 1937) and the apple (Sen 1937).

The use of nutrient solutions in such concentration and amount as to cause slight leaf damage has given successful results on a number of occasions. Its limitations as a method are suggested by an experiment already described on p. 55 in which part of the tree was permeated not sufficiently so as to cause damage, but sufficiently for subsequent growth to be increased materially.

An early experiment already mentioned (p. 37) (Roach 1934a) serves as an example showing roughly the same degree of permeation. This was recognized by the damage caused by sodium thiosulphate injected through the shoot tip, by dye injected in a similar manner, by the apparent resistance to mildew and by the increased growth following the injection of the sodium thiosulphate.

Suitable dyes may therefore be used with considerable confidence for showing the distribution likely to result from any particular type of injection. The small differences in extent of permeation due to variation in the plant and in the injected substances themselves are easily allowed for in practice.

DAMAGE TO FOLIAGE RESULTING FROM INJECTION

Damage to foliage may result from the injection of a large quantity of almost any substance or from a smaller amount if it is injected too rapidly. The character of the damage varies according to the substance (Roach and Thomas 1934) and according to the age or maturity of the leaves (Hearman, Levy and Roach 1936). The leaf symptoms so far observed have been of two main kinds; namely marginal and veinal scorching. These are shown diagramatically in text fig. 44.

Marginal damage.

Marginal leaf damage occurs only in mature leaves and shows itself as a desiccation of the edge of the blade especially towards its tip. In more severe cases this extends inwards and affects the interveinal tissue. In very dry weather the damaged tissues merely dry up, become brittle, and after a period, which varies with the weather, turn brown. More commonly the tissues become brown without going through the dried up green stage; after a time they become brittle and break away often causing the leaves to become lobed rather like oak leaves. In more severe cases the whole leaf falls. When the brown stage is reached this kind of damage is indistinguishable from that associated with potash deficiency. It was first described by Rumbold (1920), and Collison, Harlan and Sweeney (1932) suggested that the injury resulted from the fact that the injected salts reached the leaves more rapidly than they could be utilized and removed, the cells in consequence becoming plasmolized. It is produced by most of the comparatively innocuous salts such as those of potassium, ammonium, sodium, calcium and

magnesium. These facts have obvious implications as to the immediate cause of the symptoms associated with potash and other deficiencies.

Veinal damage.

The veinal type of damage illustrated in the text fig. 44 is shown by young, not fully expanded, leaves, and also by older ones that have remained immature, e.g. as a result of frost. This point will be referred to later. Such damage may be brought about by overdoses of almost any substance, and it also occurs in mature leaves when suffering from an overdose of some of the more toxic salts, e.g. those of iron salts and some organic compounds.

The most important difference between these two types of injury is that marginal damage shows itself almost immediately, whereas the veinal type may not become apparent until a week after injection.

If the injection is stopped when the first signs of marginal damage appear, there is usually no perceptible increase in it later on and it is confined to the oldest leaves. Therefore, even when heavy overdoses are given, causing many leaves to fall, the damage is not serious because the young leaves expand rapidly and replace those which have fallen.

The effects of the veinal type of damage, however, are much more serious. The youngest leaves are the most susceptible. These are often killed and, with them, the growing tip. Sometimes whole branches are killed. Its most troublesome feature is its delay in appearance; it gives no warning, as does the marginal type, that an overdose has been supplied, the result being that a fatal dose may easily be given. The importance of correct dosage is thus obvious, and this will now be discussed.

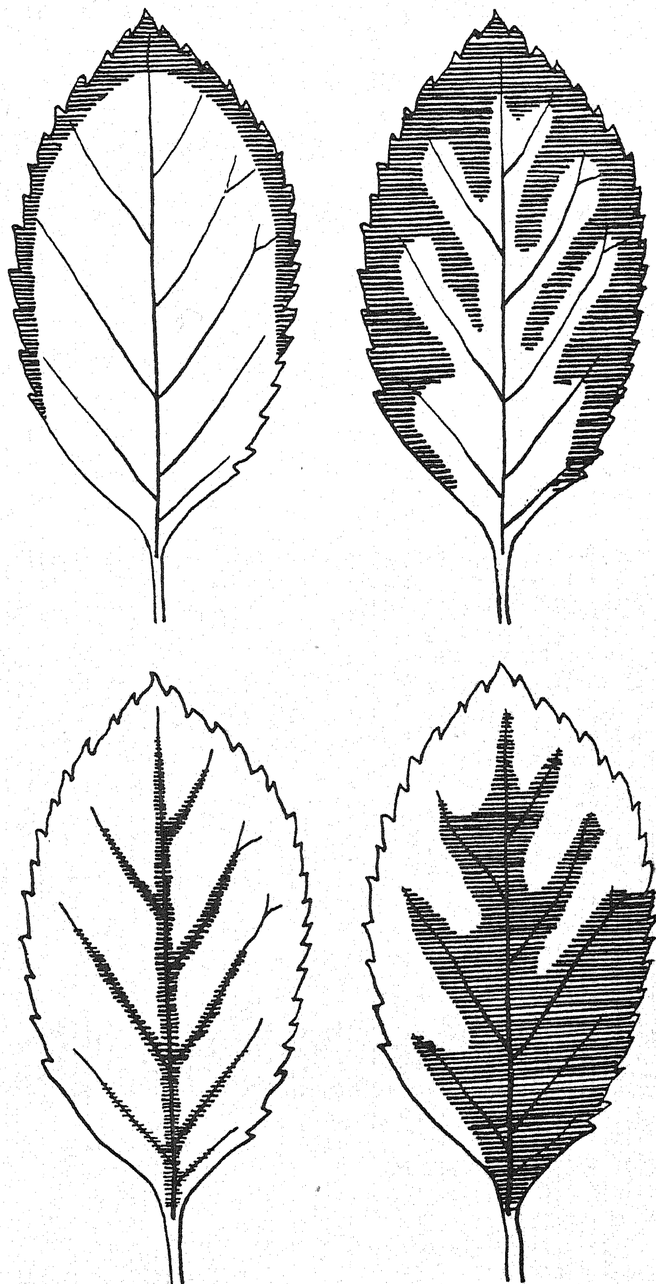


FIG. 44.
Leaf injection damage, top left marginal and right interveinal, bottom veinal left, mild—right, severe.

DOSAGE

The results of some of the early experiments suggested that good (perhaps optimum) growth responses, were obtained when the tree was allowed to absorb a $\frac{1}{2}$ or $\frac{1}{4}$% solution of the nutrient until marginal leaf damage just became apparent ; and this still seems the safest method to adopt when starting on unknown material. Great care must then be exercised to make sure that the trees will show the marginal, and not veinal, type of damage. The only safe way of deciding this question is to make a few shoot tip or other small-scale injections with, say, a 1% solution of the nutrient. If all these produce typical marginal damage which does not increase materially after injection has ceased, it is safe to proceed. A few representative trees are then injected with the concentration decided upon and examined every few hours for leaf damage. When such damage is noticed injection is stopped and the volume of injected liquid is noted. This, or a slightly smaller volume may then be given to similar trees which may be allowed to become injected with it without being watched so closely.

When an iron salt, or some other substance which produces veinal leaf damage, is to be injected, it is sometimes worth while injecting one or more trees with, say 0·25% potassium sulphate until marginal damage is produced, and then to inject an equal volume of a ~~0·25%~~ 0.025% solution of the iron salt. Even if, as often occurs, a little leaf damage is produced by the iron solution, it will not be serious because the growing points of the leaves are not damaged unless a heavy overdose is given.

When, however, veinal damage is caused by the original small-scale test injection, great care must be exercised because the growing points then tend to be killed by quite small overdoses. Veinal damage commonly results from injection of leaves that are thin, rather light in colour and " unfinished " in appearance. Frost, when the buds are bursting, or even more so when the leaves are expanding, seems to be the usual cause of this apparent arrested development of leaves. Varieties of apples differ in their susceptibility to this trouble ; Lane's Prince Albert is particularly prone to it and Bramley's Seedling is comparatively resistant. Immature leaves of this kind do not appear to respond to injection ; it is therefore useless to attempt diagnostic injection early in such seasons as that of 1937, i.e. when leaves show arrested development. When possible it is best to wait until some normal leaves have become fully expanded before attempting large scale injections. When these have to be carried out without waiting for the most suitable moment it is usually safe to inject 0·25% solutions of the ordinary nutrients for half a day if both the air and soil are dry, and for a whole day when injection conditions are not so good.

Results to be published shortly by Levy suggest that the cross-sectional area of stem, whether of a whole tree or of a branch, may be used as a basis for the calculation of dosage, but results obtained by Hill (also to be published shortly) suggest that a branch or tree making vigorous extension growth may be injected without harm with larger amounts than one of equal cross-section area making less extension growth.

LOCALIZATION OF EFFECTS OF INJECTION

The value of all the methods for injecting parts of plants, in fact all those described in this paper except that for injecting whole trees, depends on the conducting channel for various parts of the plant being to a certain extent independent. Auchter (1923) concluded from his own experiments and from those of others, that the mineral nutrients absorbed by the roots on one side of a plant are in large measure used by the leaves in direct connexion with them, and that there is apparently very little transfer of such nutrients from one side of the plant to the other. As was shown in an early paper by the present writer (Roach 1934a), if a tubular glass contrivance such as that shown in text fig. 45 be made and supposed to represent a tree with its roots, stem and branches, and if the two lower " roots " be dipped into two dishes containing differently coloured liquids of the same density, then when uniform suction is applied to each branch as through the black rubber tubing shown, the liquids will travel up the central

tube in parallel columns which do not mix. The columns pass into the branches at the upper fork, still without mixing. The liquid in each branch may be all of one colour or be of two colours, side by side, without lateral mixing. The pull of the leaves is represented by that of the curved rubber tubes acting as siphons, from the ends of which the liquids are allowed to discharge at uniform rates down a piece of cotton to prevent the slight irregularity of movement if the liquid is allowed to drop from the ends of the tubes. Freedom from vibration is essential for success of the experiment. With this apparatus unequal soil moisture in the two roots and unequal transpiration in the two branches may be imitated by constricting a "root" or "branch" rubber tube by means of a screw clip. There is no mechanism in a tree, tending to mix these streams, on the contrary the structure of plants tends to prevent such mixing.

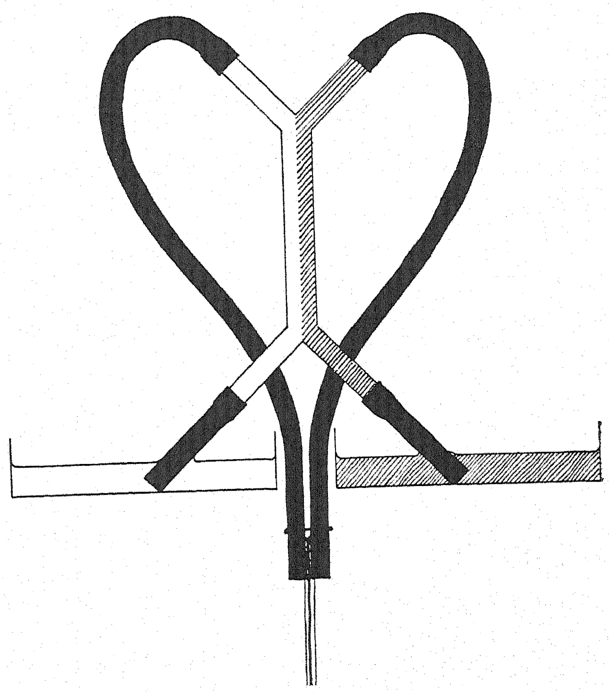


FIG. 45.
For description, see text.

Thus in numerous early experiments leafy trees of various sizes were lifted and the cut end of a single root of each was immersed under one dye solution and the remaining roots were immersed in another dye solution. In each experiment the dye could be traced up the strands in the main stem in direct connexion with the single root into the leaves at their other end. There was never any mixing of the dyes. Even when only a single root was immersed in dye, the remaining roots being allowed to dry, the dye ascended the strands in direct connexion with it for a distance of many cm. before any movement into contiguous strands could be detected. A similar result was obtained when the cut end of a branch was immersed. The dye descended the strands in direct connexion with it a distance of many cms. before invading neighbouring strands and entering the leaves in connexion with them.

If any is required perhaps the best justification for the employment of plant injection methods are the results already obtained with them. These include the following: (i) The effects of interveinal injections have been confined to the injected areas until the leaves have fallen naturally in the autumn. (ii) The injection of iron sulphate in the autumn of 1935 into shoot tips of chlorotic cherry trees resulted in the turning green of a number of leaves at the end of injected shoots. These remained green not only during the summer of 1936 but the same shoots were green in the autumn of 1937, still in striking contrast with the rest of the tree. (iii) The effects of injections of branches have remained confined to those branches for a whole season.

It would be premature to enquire how long the effects of injection remain localized; the above facts open up a sufficiently wide field of usefulness for the present; but experiments to answer the question are contemplated.

INJECTION OF PLANTS WHEN LEAFLESS

Injection in early winter, though much less vigorous than in summer, is still considerable. The following experiments were carried out to compare the results of summer and winter injection, and to determine to what extent winter injection is dependent on transpiration.

Two straight two-year-old leafless apple shoots were found growing on the same branch of the same tree two inches apart, the current year's growths of which were practically equal in length and diameter. Of these one was cut off at 10 a.m. on 17/4/34, near the branch from which it arose and was then sawn through under water at the junction of the current growth with the previous year's. The current year's growth was held in an inverted position under water and its cut end was connected by stout rubber tubing to a glass tube having the same diameter as the stem at its lower end, but constricted at the upper, as shown in text fig. 46, a mark having been made on the constricted part. The shoot with tube attached, after being dried with a cloth was suspended by a wire hook to one arm of a balance, the tube filled to the mark with water and the whole weighed. Periodical weighings were made, the tube being filled to the mark each time and the whole re-weighed. From the figures obtained, the weight of water lost by the twig and the weight of water added were calculated. Water lost by evaporation from the free end of the glass tube was found to be negligible. The weight of water added was at first many times that lost, hence a large proportion of the water absorbed by the shoot was retained in its tissue. The amount of water thus retained decreased gradually until at the end of five days it was negligible.

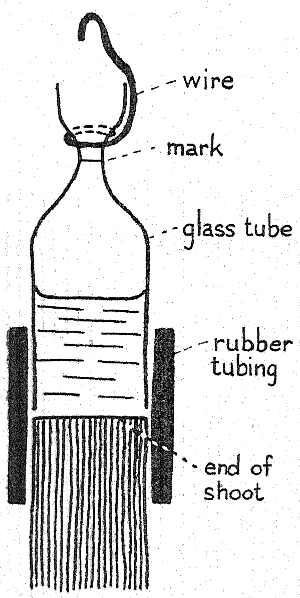


FIG. 46.
For description, see text.

The results are represented graphically in curves AT and AR in text fig. 47. Transpiration losses were too small to be estimated in the first few hours when weighings had to be frequent because of the steepness of the retained water curve. The weighings were continued until retention became negligible, then the shoot was disconnected from the tube and placed with its cut end in water.

At the end of two months roots had formed at the base of the shoot and leaves were just expanding. The experiment was repeated with the same shoot after half an inch had been cut from its base. The results are shown in curves BT and BR. Transpiration was slightly greater than when the shoot was quite dormant, but the retention curve was practically identical with the original one. The shoot, therefore, must have closed the vessels through which it was originally injected and reverted to its original state, resembling that of a partially evacuated vessel.

At 10.25 a.m. on 12/6/34 the second shoot, now with leaves fully expanded, was cut from the tree and treated as the first. Curves CT and CR show that transpiration was enormously greater than for the dormant shoot; but that retention was again practically the same as for it. The total weight of retained water in each of the above experiments was about 2·5% of the total weight of the shoot.

In a somewhat similar experiment, carried out on a dormant four-year-old plum branch

in December 1932, the retained water was about 4% of the total weight of the branch. This experiment differed from those on apple shoots in that the branch was suspended with its cut end downwards. The water into which the cut end dipped was held in a small flask fixed rigidly to the branch; the water, therefore, entered the branch against the pull of gravity.

The above experiments suggest that only a limited volume of liquid can be injected into a cut shoot or a lifted tree when leafless, whereas when there are leaves on it the amount is practically unlimited. The liquid enters tissues of the leafless shoot which behaves like a partially evacuated vessel; as soon as this is filled no more can enter. In the leaf shoot, on the other hand, not only are these partially evacuated tissues filled, but water is continually removed by transpiration, and more can enter to take its place. Another respect in which liquid injected into a leafless tree behaves like liquid entering a partially evacuated vessel is the fact that its rate of advance becomes less and less as the shoot tip is approached. When a whole tree is injected, the current year's extension growths do not become permeated until the leaves expand and draw the liquid along them.

These few remarks will suggest the limited possibilities of injecting leafless plants, but the greater water content of the woody tissues in winter than in summer may perhaps be an advantage when the solid injection method is used.

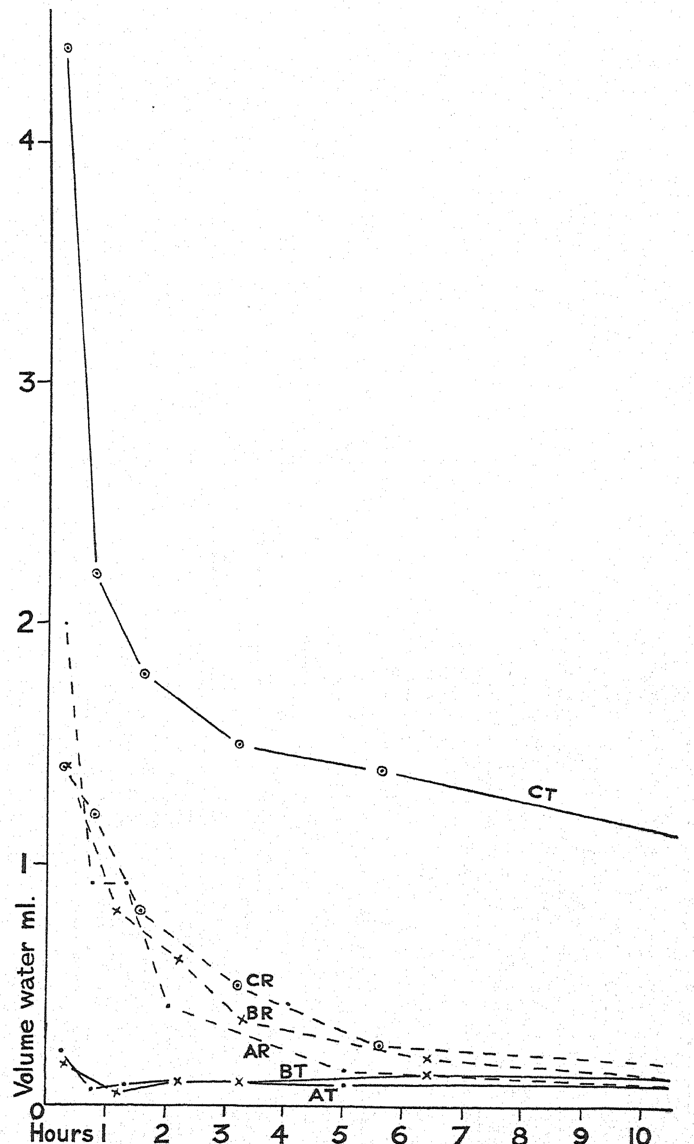


FIG. 47.

Relative amounts of water, absorbed by an apple shoot in the dormant and growing seasons, that are retained in the shoot and lost from it by transpiration.

AT Loss of water per hour by transpiration.
AR Water retained per hour by a dormant shoot.
BT Loss of water per hour by transpiration.
BR Water retained per hour by the above shoot after its cut end had stood in water for two months. Buds just expanding. Fresh cut made ½″ from original one.
CT Loss of water per hour by transpiration.
CR Water retained per hour by a leafy shoot of equal size just cut from the tree.

SOLID INJECTION

In the course of the present work solid substances have been used for injection on a few occasions only. Solutions must obviously be used for all the leaf injection methods ; and their more rapid distribution make them more convenient for working out general principles. Mention has already been made of some of the results obtained by other workers with the solid method, particularly when applied to whole trees, and sometimes even on a large commercial scale. In view of the many factors already noted which tend to influence the injection and distribution of the liquid used, it has seemed unwise up to now to attempt any comparison between the solid and liquid methods in circumstances in which both are applicable ; but such comparisons should soon be possible. Only a few tentative remarks can be made at present. Leaf injection methods, feasible with liquids alone, include the quickest for diagnostic purposes. Solids cannot be used for the shoot tip method and hardly for the branch methods, with small branches. For larger branches and whole trees, the response to solid injection appears to be slower than that to liquid injection ; circumferential movement seems less from a solid than from a liquid injection for the former. Nevertheless, the fact that no liquid is necessary for a solid injection is definitely in its favour for commercial use. Further work is obviously necessary before the best methods for all purposes can be decided on and a number of possible modifications remain to be tested.

GENERAL APPLICATION OF PLANT INJECTION METHODS

Having now described the main types of distribution of the introduced substances obtainable by injection and having mentioned some of the results already obtained by these methods, one may recapitulate in more general terms the fields of work in which plant injection is most likely to be useful.

The results mentioned in this paper alone are enough to suggest that each of the common nutrients produces the same effect on a plant, whether it is injected artificially or is absorbed by the roots in the ordinary way, and that consequently the artificial mode of supplying the test nutrients by injection does not militate against the use of results so obtained for diagnosing mineral deficiencies.

By one of the methods described a single interveinal area may be injected with a test liquid, which becomes so distributed that the treated area is separated from the untreated areas on each side of it by a secondary vein ; this separation is so sharp that the slightest change in colour or texture of the treated area is easily detected, permitting mineral deficiency to be diagnosed in a week, or even less. Further, the occurrence of single interveinal areas, healthier than the rest of the leaf, is so uncommon in untreated leaves that the probabilities are a thousand to one against the response shown by a single treated area being due to chance ; and if the experiment be carried out in duplicate the odds become a million to one, a degree of certainty rarely achieved in experimental work on plants.

An equally rapid diagnosis may be obtained, with the same degree of freedom from interference from natural variation, by the leaf stalk method of injection, which, when applied to apples and many other plants, results in a number of selected leaves becoming injected on one side of their midrib but remaining untreated on the other. These two methods can be carried out quickly and entail only negligible interference with the tree or plant, consequently they seem suited both for experimental trees and for those growing in commercial plantations. They appear to hold out the possibility of diagnosing not only gross mineral deficiency but also the early stages of such deficiencies. In consequence it should be possible to avoid the worst manifestations and adverse results of mineral deficiency by correcting the tendency at an early stage, and also to maintain trees in a much higher condition of health than is possible at present.

As already noted, it took no more than two hours to carry out the injections which demonstrated the widespread occurrence of incipient chlorosis of the iron shortage type in the Research Station plantations (Roach and Levy 1937). This work was done to test the suspicion, entertained for some years, that a lowered content of organic matter in the soil was leading to a

diminished availability of iron and possibly of other elements. Probably this applies to wide areas under fruit cultivation. The remedy, in all probability, should be a change in cultural practice to increase the soil organic matter, but while this somewhat slow-acting cure is being employed the more seriously affected trees can be cured rapidly by injection. Injection also seems to be the only cure which will act quickly enough to save considerable numbers of chlorotic fruit trees growing in excessively calcareous soils.

The soils of wide areas of the world have become seriously impoverished of organic matter and, as a consequence, the mineral salts previously held by the organic matter have been leached out. It is doubtful whether the mere replacement of the organic matter alone will ever result in the conversion of enough of the salts from the solid soil particles to a more available form, and it is still more doubtful whether this will occur in a reasonable time. The problem will have to be faced of first determining what minerals are deficient in quantity and secondly of replacing deficiencies either as a temporary or as a permanent measure. Injection is probably the simplest diagnostic method and it is worth considering as a practical cure in exceptional circumstances.

The shoot tip, branch and whole tree injection methods are neither as sensitive nor as rapid as the two leaf methods, but they may be used for studying effects on gross growth response and on yield and quality of fruit. The value of all these methods in general fruit advisory work is being tested by Messrs. W. G. Kent, Thompson and Duggan in co-operation with the writer throughout the county of Kent. The methods are also being tested on a variety of crops by other workers in this country and in many overseas areas.

The drift soils on which fruit is largely grown in the south-eastern counties of England vary so markedly, even within short distances, and their manurial treatment, which has often been very heavy, has varied so greatly from place to place that the more detailed manurial problems can be solved only by experiments carried out in the commercial plantation itself. The degree of randomized replication necessary in manurial experiments devised to deal with such problems, as well as their cost and inconvenience, make them inapplicable to commercial plantations. This therefore appears to be a great field of work for which injection methods may well be suited.

For these, and other types of problems to be mentioned later, injection methods seem likely to have a theoretical and later a practical, advantage over even the best planned manurial experiment. It is well known that when a substance, such as copper salt, is applied to soil, the greater part of the copper, even 99% is precipitated or absorbed into the solid part of the soil, while there is liberated into the soil solution an equivalent amount of a number of other elements. The observed effect on the plant by its application to the soil may be due either to the 1% of the copper salt left in solution or to any of the substances liberated or to both. Suggestive facts are supplied by the Broadbalk experiments at Rothamsted where wheat has been grown continuously for a large number of years under various manurial treatments. The two plots receiving equivalent amounts of potassium and sodium salts, respectively, gave equally good crops for several years, and both were better than the unmanured plot. But after more than ten years the yield of the plot receiving sodium salts fell behind that of the one receiving potassium salts. The reason proved to be that at first the sodium displaced potassium from the soil until all the readily replaceable potassium had been used ; then the sodium, which is unable to perform the same function as potassium in the plant, was no longer effective and the yield fell. The condition of land such as that of the sodium plot is doubly unsatisfactory. Not only does it now yield badly, but before applications of potassium salts can have their full effect on the crop all the sodium already absorbed into the solid part of the soil must be replaced by potassium, since until this has been done the whole of the potassium applied will not be available to the plant. This well established and classic example is likely to be far from an extreme case ; in fact, other elements, such as copper which has just been mentioned, become absorbed and retained much more tenaciously by the solid soil particles than either sodium or potassium. One instance of the practical importance of copper salts has already been given. Another has recently been discovered by Teakle and Dunne (Teakle 1937, and Dunne 1938) in Australia by the use of injection methods. He found that a serious dieback disease could be cured by injecting a copper salt. Later he found that soil application was effective. The work of Allison and his co-workers (1927) has shown that copper, manganese or nickel can each be of great benefit to crops on an exceptional

type of peat soil. There is increasing evidence that the functions of most, if not all, of the elements are quite specific and that one element cannot replace another. It is tempting to speculate, therefore, whether the similar immediate, practical value of these three elements is not really due, at least in part, to base exchange of the type described above. If this be so the determination of which elements are actually used by the plant is likely to be one of the utmost practical importance in the not distant future. Such a problem can be solved quickly and simply by injection methods.

There is a far reaching problem of this type of immediate practical importance in fruit growing. Fruit trees, in general, require large quantities of potassium, and it is a common experience that large quantities must be added to the soil before any appears to reach the tree, and some years elapse before there is any improvement in its condition. In such circumstances the injection of all trees in a plantation to make sure of attaining an immediate response may well be the most practical solution. At the same time, or later, the systematic treatment of the soil can be taken in hand.

The cause and cure for a disease of apples of great economic importance, especially in Canada and New Zealand, has recently been discovered by injection methods. Atkinson (1935) in New Zealand, McLarty (1936) in British Columbia and Young and Bailey (1936) in New Brunswick, working independently, each found as a result of injecting a number of substances into apple trees, that boron compounds alone cured the trouble. A little later Jamalainen (1936) confirmed this discovery by experiments carried out in Finland. These workers have determined suitable dressings for application to the soil and there is consequently a commercially practicable cure for the trouble on most soils. Calcareous soils still, however, present difficulties (Hill 1937) and until these are overcome the disease may perhaps be cured by injection. The disease has been known by various names e.g., Corky Core, Drought Spot, etc., and shows a number of widely different symptoms which have been confused with others similar in appearance, but evidently due to a different cause. It is now possible to group together all those symptoms cured by boron (Hill 1937) and, having verified by injection experiments the fact that the remainder are not thus curable (Levy and Roach 1937) it is proposed to seek for cures for the remainder by similar methods but by using different compounds, guidance as to what substances to try being obtained by chemical analysis of healthy and affected plants.

In experiments already described (Roach 1935a) the injection of apple trees with a solution containing dipotassium hydrogen phosphate and urea was followed by a marked increase in vigour; the treated trees were free from two insect pests, leaf hoppers (*Jassidae*), and red spider (*Oligonychus ulmi* Koch), in marked contrast to the untreated trees which were heavily infested; and as already stated (p. 5) some unpublished results obtained by H. Wormald and the writer suggest that a fungus disease, silver leaf, may also be controlled by the injection of nutrient salts or other substances. Similar methods offer a comparatively simple means of determining how far insect and fungus diseases can be controlled by adjusting the nutrition of the host.

In the experiments just mentioned the injection of a disinfectant, either alone or with nutrients, was found to control this fungus disease and experiments have been mentioned earlier in this paper (p. 37) in which apple mildew (*Podosphaera leucotricha*) was controlled by injecting sodium thisulphate. Further, the results of preliminary experiments carried out by R. M. Greenslade suggest that an insect pest, woolly aphis (*Eriosoma lanigerum* Hausmann), can be controlled by the injection of an insecticide, a conclusion in harmony with that of Dementiev (1914).

Only the first of the three observations noted above is of any immediate practical value, but the three together suggest that systematic development of work of this kind might well give valuable results.

It is unnecessary here to discuss at any length the possible uses of injection methods in plant physiological research. One problem will be mentioned because it actually led to the work which has just been described. It is well known that the rootstock on which a fruit tree is budded or grafted exerts a marked effect on the size and vigour of the tree, on its resistance to various insect and fungus diseases, its precocity or lateness in starting to bear fruit, on the season at which this fruit matures, its colour and keeping quality, and in a variety of other ways. Many of these characters are of great commercial value and one of the main aims of pomologists is to combine

in a single tree all the good qualities scattered amongst a number of varieties and rootstocks. The fact that soil differences and artificial manuring may give similar (but not quite identical) effects to those of rootstock, led to the discovery that the rootstock, in common with them, influenced the mineral composition of the top of the tree, and it became desirable to test the effects on the tree of each of the twenty odd elements which occur in it—most of them in minute amounts (Roach 1931). What has already been written will suggest the advantages that injection methods should have for this purpose.

Two other fields of work are simplified by the fact that fruit itself may be injected either while still on the tree or even after it has been picked. In this way its content of desired constituents may be increased at will without seriously affecting the amounts of other constituents. This can hardly be done by any method other than injection. In colloboration with Mr. Levy and the writer, Dr. Hulme of the Ditton Laboratory is studying the effect of such variations of composition on respiration and keeping quality, and Dr. A. S. Horne of the Imperial College of Science, London, is studying their effect on resistance to fungal invasion.

SUMMARY

1. A review is given of the history of plant injection, which goes back at least to the twelfth century.

2. Methods are described for injecting individual parts of a plant each with a different liquid, using the untreated parts for comparison.

3. The main purpose of these methods is the diagnosis of mineral deficiencies.

4. Such diagnosis may be carried out in as short a time as four days.

5. Some of these methods have a high degree of statistical reliability.

6. Single interveinal areas of certain plants (apple, pear, strawberry, etc.) may be injected so that the permeated area is separated sharply by a secondary vein from the untreated area on either side. This method is not applicable to the peach and the tomato.

7. A leaf after its apical quarter has been amputated may be injected while still attached to the tree by immersing the cut edge.

8. Similar treatment of the leaflet of a compound leaf in some plants (e.g. the strawberry) results in the permeation of one side of the adjacent leaflet, while the other side is not permeated.

9. Injection, through a leaf stalk left attached to the stem after the blade has been removed, results in the permeation of whole leaves and parts of leaves above and below the injection point, the injection pattern depending on the phyllotaxis and the vascular anatomy of the stem. In some leaves the midrib forms a sharp division between permeated and unpermeated blade tissues.

10. Injection through a shoot tip results in the permeation of a number of the terminal leaves.

11. Branches may be injected independently of the rest of the tree through a suitably placed hole passing diametrically through the branch.

12. The individual branches of certain trees may be injected each with a different liquid so that the corresponding roots are also permeated.

13. Whole trees and other plants may be injected through a single diametrical hole, large ones through two or more radial holes.

14. Uniform permeation of all the branches depends on the proper placing of the hole or holes in relation to the branches.

15. Examples are given of experimental or practical applications of all these methods.

ACKNOWLEDGMENTS

Much help has been acknowledged at various places in this paper. The writer wishes to thank Mr. D. Akenhead, Professor V. H. Blackman and Dr. G. H. Pethybridge for many valuable suggestions concerning the presentation of this paper and to Mr. D. Akenhead for seeing it through the press during the writer's absence in South Africa.

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